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A System for Estimating Drug Delivery from a Dry Powder Inhaler by Analysis of Acoustic Recordings of Time-Stamped Inhaler Events

Jansen N. Seheult
Royal College of Surgeons in Ireland, jansenseheult@rcsi.ie

A System for Estimating Drug Delivery from a Dry Powder Inhaler by Analysis of Acoustic Recordings of Time-Stamped Inhaler Events

A dissertation submitted to the
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By

Jansen Seheult, MB BCh BAO (NUI, RCSI), MSc (Dubl.), LRCP&SI
Department of Medicine Respiratory Research Division,
Royal College of Surgeons in Ireland,
Dublin, Ireland

Research carried out under the supervision of:

Professor Richard Costello,
Department of Medicine Respiratory Research Division,
Royal College of Surgeons in Ireland,
Dublin, Ireland

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Candidate Thesis Declaration

I declare that this thesis, which I submit to RCSI for examination in consideration of the award of a higher degree Doctor of Medicine is my own personal effort. Where any of the content presented is the result of input or data from a related collaborative research programme this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own. I have not already obtained a degree in RCSI or elsewhere on the basis of this work. Furthermore, I took reasonable care to ensure that the work is original, and, to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

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List Of Abbreviations

BMI	Body Mass Index
cAMP	Cyclic Adeonsine Monophosphate
C_{max}	Maximum concentration
COPD	Chronic Obstructive Pulmonary Disease
CV	Coefficient of Variation
CYP	Cytochrome P_{450}
DPI	Dry Powder Inhaler
DUSA	Dosage Uniformity Sampling Apparatus
ELISA	Enzyme Linked Immunosorbent Assay
ESI	Electro-spray Ionization
FA	Formic Acid
FBE	Filter Bank Energy
FEV1	Forced Expiratory Volume in 1 Second
FFT	Fast Fourier Transform
FIVC	Forced Inspiratory Vital Capacity
FPD	Fine Particle Dose
FPF	Fine Particle Fraction
FVC	Forced Vital Capacity
GINA	Global Initiative for Asthma
GOLD	Global Initiative on Obstructive Lung Disease
GSD	Geometric Standard Deviation
HPLC	High Performance Liquid Chromatography
IC	Inspiratory Capacity
ICH	International Conferences on Harmonisation
ICS	Inhaled Corticosteroids
Ig	Immunoglobulin
IL	Interleukin
INCA	Inhaler Compliance
IS	Internal Standard
LABA	Long Acting Beta Agonist
MA	Median Amplitude
MAD	Mean Absolute Deviation

MDI	Metered Dose Inhaler
MFCCs	Mel Frequency Cepstral Coefficients
MMAD	Mass Median Aerodynamic Diameter
MS	Mass Spectrometry
NGI	Next Generation Impactor
NMD	Neuromuscular Disease
NRC	Non-respiratory Condition
P_{ave}	Average Power
PEFR	Peak Expiratory Flow Rate
PET	Polyethylene Terephthalate
PIFRc	Peak Inspiratory Flow Rate (calculated)
PIFRm	Peak Inspiratory Flow Rate (measured)
PKA	Protein Kinase A
pMDI	Pressurized Metered Dose Inhaler
PSD	Power Spectral Density
RMS	Root Mean Square
ROC	Receiver Operating Characteristic
SABA	Short Acting Beta Agonist
TED	Total Emitted Dose
Th2	T-helper 2
T_{max}	Time to maximum concentration
UAD	Upper Airway Dose
Ultra-LABA	Ultra-Long Acting Beta Agonist
UV	Ultra-violet detection

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Presentations And Publications

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Summary

Inhaled medications are the mainstay of therapy in the treatment of chronic respiratory diseases like asthma and COPD because they allow delivery of the active ingredient directly to the site of action. Poor adherence to inhaled controller medications has been estimated to account for up to 60% of asthma-related hospitalizations and increased rates of 30- and 60- day hospital readmissions in patients with COPD. Numerous electronic monitoring devices have been developed over the last four decades to monitor temporal non-adherence; however, many of these devices do not monitor all or most aspects of inhaler technique. Currently used methods for monitoring inhaler technique, including subjective checklists, are suboptimal.

There is a need to study the frequency of temporal and technique non-adherence in the Irish population and to investigate the impact of dosing and technique errors on drug delivery. Moreover, a comprehensive system of tracking the date and time of inhaler use, as well as the presence or absence of technique errors, on a daily basis is essential to not only an epidemiological understanding of inhaler use but to tailoring of inhaler training and clinical care plans to individual patients. This thesis describes the use of the INCA^{TM} device, a novel acoustic monitor, which provides longitudinal data on the date and time of inhaler use, as well as data on inhaler technique.

Studies showed that inhalation flow rate, exhalation into the inhaler mouthpiece prior to inhalation, breath-hold duration and missed doses had a significant effect on delivered dose. Data on both temporal and technique adherence were combined in an algorithm, which provided a single measure of overall adherence, called “actual adherence”. The dose counter rate correlated poorly with INCA^{TM} derived adherence rates, highlighting the need to incorporate technologies, like the INCA^{TM} device, into clinical trials and patient care.

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Dedication

I dedicate this thesis to my parents, who have made tremendous sacrifices to allow me to pursue my career goals. They have provided unwavering support and I owe where and who I am today to them.

I would also like to dedicate this thesis to my girlfriend, Portia, who had the unenviable task of putting up with my steady stream of complaints and frustrations when experiments failed and who helped me maintain composure during many trying times.

CHAPTER 1 – Introduction

1.1. General Introduction

Asthma and chronic obstructive pulmonary disease (COPD) are two common types of chronic respiratory disease, characterized by airways obstruction. Prevalence of these two respiratory diseases has risen sharply over recent decades, creating a large economic burden due to treatment costs.(1, 2) Prevalence estimates for COPD range from 7.6% to 34.1% depending on the spirometric criteria used for diagnosis and in 2010, COPD became the third leading cause of death worldwide.(3, 4) Estimates for asthma prevalence vary widely from approximately 1-4% in developing countries to as high as 20% in developed countries.(5, 6) Exacerbations of COPD and asthma constitute the main component of the burden of disease, often leading to increased physician visits, hospitalizations or death. The global burden for patients from asthma exacerbations and day-to-day symptoms has increased by almost 30% in the past 20 years.(7) The total direct costs of respiratory disease in the European Union is estimated at more than 6% of the total health care budget, with COPD accounting for more than half of this amount.(8) In 2010, the direct costs attributable to COPD was estimated at $32.1billion in the United States, with hospital admissions being the largest contributor.(9)

1.1.1. Asthma

Asthma has traditionally been thought of as a chronic inflammatory disease of the airways, characterized by variable and recurring symptoms of wheeze, cough, dyspnea and chest tightness, reversible airflow obstruction, airway hyper-responsiveness and bronchospasm, and airway inflammation.(10) Bronchial hyper-responsiveness is provoked by numerous triggers, including exercise, viral upper respiratory tract infections, cigarette smoke and respiratory allergens.(11) Hyper-responsiveness is tested clinically by monitoring lung function parameters after provocation with methacholine or histamine.(12)

Immunologically, asthma is characterized by a T-helper subclass 2 (Th2) response with production of Interleukins (IL) 4, 5 and 13, a switch from

immunoglobulin (Ig) M to IgE, mast cell degranulation and recruitment of eosinophils to the airways.(11) It is now also recognized that some asthmatic inflammation is neutrophilic in nature and controlled by T-helper 17 cells.(13) Asthma has now been dichotomized into Th2-high and Th2-low endotypes based on the role of type 2 T-helper cells in the pathobiology of the disease.(14)

Chronic inflammation in asthma leads to “airway remodeling” with alterations in the airway epithelium, lamina propria and submucosa, leading to airway thickening.(15) Grossly, there is lung hyperinflation, smooth muscle hypertrophy, mucosal edema, mucus gland hypersecretion, epithelial sloughing and ciliary dysfunction.(16)

Establishing a diagnosis of asthma depends on medical history and physical examination to show that symptoms of recurrent episodes of airflow obstruction are present. These symptoms include wheeze, cough, chest tightness or dyspnea and usually occur or are worsened by triggers such as viral infections, allergens like the house dust mite or pollen, irritants like tobacco smoke, changes in weather, stress, exercise or strong emotional expression.(17) The clinician should also consider and rule out other causes of airway obstruction. Confirmation of reversible airflow obstruction is made by spirometric or lung function tests in patients older than 5 years of age.(17)

1.1.2. Chronic obstructive pulmonary disease (COPD)

Chronic obstructive pulmonary disease (COPD) is characterized by progressive airflow restriction that is not fully reversible, associated with abnormal airway inflammation in response to noxious particles or gases and systemic effects, such as ischaemic heart disease, heart failure, diabetes and lung cancer.(18) In fact, the major burden of morbidity and mortality in COPD is related to the extra-pulmonary effects.

Cigarette smoking is the major cause of COPD leading to direct injury of airway epithelial cells, release of inflammatory mediators, reactive oxygen species and proteolytic enzymes, as well as impaired immune regulation and immunological senescence.(18) The chronic airway inflammatory response results in emphysema due to parenchymal tissue destruction, loss of alveolar attachments and decrease in elastic recoil; and small airways disease due to airway fibrosis and luminal plugs, in turn leading to air trapping and progressive airflow obstruction.(19)

The diagnosis of COPD is also based on a thorough clinical history and physical examination, including assessing for extrapulmonary disease, but spirometry plays a significant role in establishing a diagnosis.

1.1.3. Spirometric diagnosis of asthma and COPD

Mechanical respiratory abnormalities can be classified into obstructive or restrictive defects based on the presence or absence of flow-related or volume-related defects.(20) Asthma and COPD are examples of obstructive airways diseases. The diagnosis of COPD must include an FEV1 (forced expiratory volume in 1 second) to FVC (forced vital capacity) ratio of less than 0.70.(21)

The Global Initiative on Obstructive Lung Disease (GOLD) classifies COPD into four stages based on post-bronchodilator FEV1% predicted (22):

Stage 1: Mild, FEV1>=80% predicted
Stage 2: Moderate, FEV1 50-80% predicted
Stage 3: Severe, FEV1 30-50% predicted
Stage 4: Very severe, FEV1 <30% predicted

Asthmatic patients may or may not exhibit an FEV1/FVC ratio less than 0.70 but should demonstrate evidence of reversibility, that is, an increase of at least 12% or 200ml in FEV1 after administration of a bronchodilator.(21)

It is now recognized that there may be some spirometric overlap between asthma and COPD, since some COPD patients may show a degree of reversibility in FEV1 after bronchodilator therapy, although the FEV1/FVC ratio usually remains less than 0.70 in this group.(23) Hence, clinical history and physical examination remain the key to distinguishing between COPD and asthma.

1.1.4. Management of asthma

In recent years, the foundation of asthma management was based on determining the degree of severity or intrinsic intensity of the disease process and level of control.(17) Asthma has been classified into and managed in a stepwise fashion based on the following categories(10):

1) Intermittent:
- Symptoms ≤ 2 days/ week
- Night-time awakenings ≤ 2nights/ month
- Use of Short acting beta agonist (SABA) ≤ 2 days/ week
- No interference with normal activity
- Lung function: Normal FEV_1 (> 80% predicted) and normal FEV_1/FVC ratio
- Treatment with as needed SABA

2) Mild Persistent:
- Symptoms > 2 days / week but not daily
- Night-time awakenings ~ 3-4 nights/ month
- Use of short acting beta agonist (SABA) > 2 days / week but not daily
- Minor limitation on normal activity
- Lung function: Normal FEV_1 (> 80% predicted) and normal FEV_1/FVC ratio
- Treatment with low dose inhaled corticosteroids (ICS) and as needed SABA

3) Moderate Persistent:

- Symptoms daily
- Night-time awakenings > 1/ week but not nightly
- Use of short acting beta agonist (SABA) daily
- Some limitation on normal activity
- Lung function: FEV_1 > 60% but <80% predicted and FEV_1/FVC ratio < 0.7
- Treatment with low dose ICS + long acting beta agonist (LABA) and as needed SABA

4) Severe Persistent:

- Symptoms throughout the day
- Night-time awakenings nightly
- Use of Short acting beta agonist (SABA) several times daily
- Extreme limitation on normal activity
- Lung function: FEV_1 < 60% predicted and FEV_1/FVC ratio < 0.7
- Treatment with medium-high dose ICS + LABA and as needed SABA with consideration of Omalizumab and oral corticosteroids

The new definition of asthma according to the revised Global Initiative for Asthma (GINA) guidelines describes asthma as a “heterogeneous disease, usually characterized by chronic airway inflammation…defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity…with variable expiratory airflow limitation.”(24) The new GINA guidelines also stress the need to individualize patient management, by considering behavioural, social and cultural factors.(24, 25)

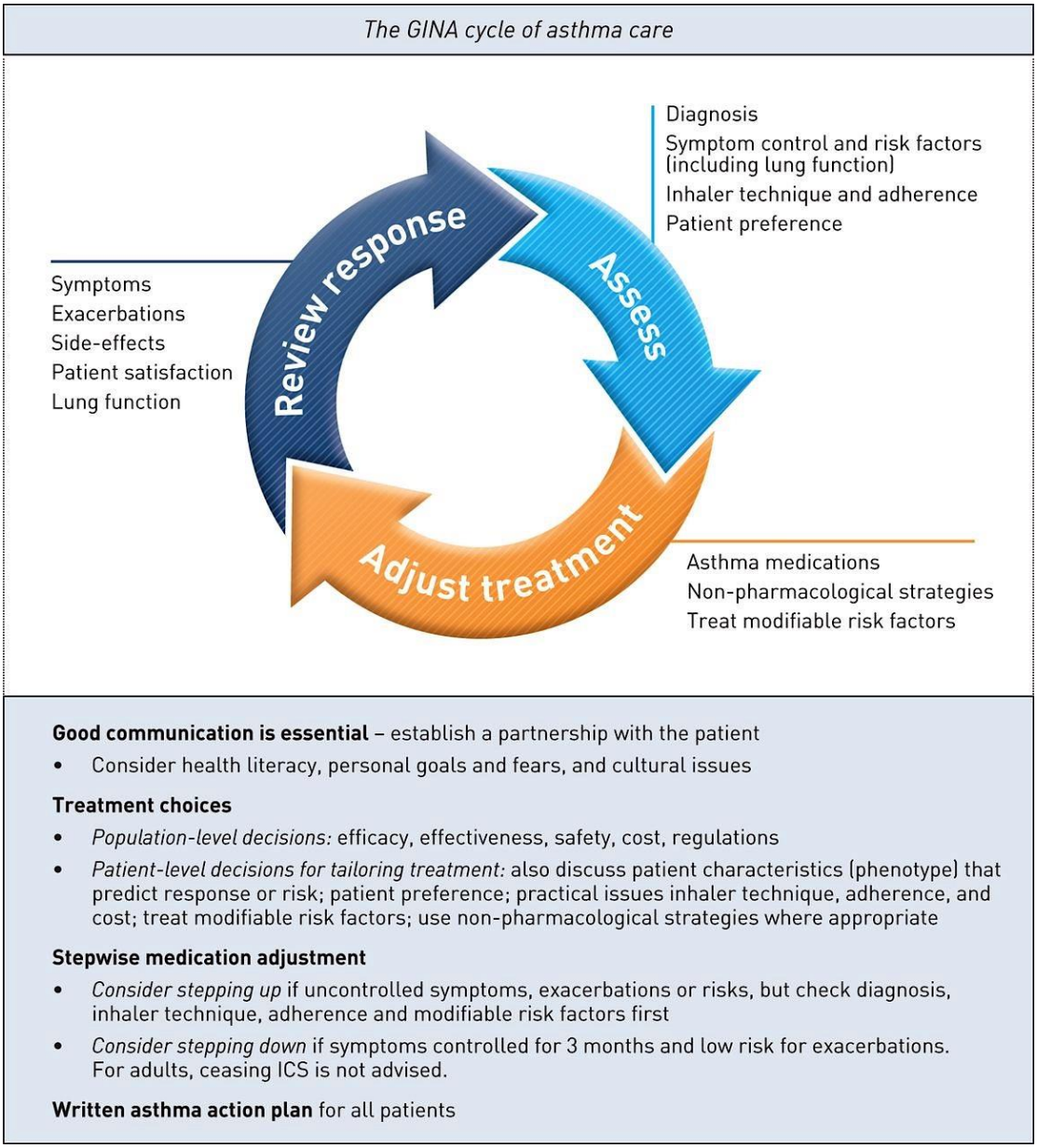

Figure 1.1: Steps involved in asthma diagnosis, management and treatment. Reproduced from (26). ICS: inhaled corticosteroids.

Figure 1.1, taken from the GINA 2015 report, highlights a new control-based care system for treatment of asthma. One key point stressed in the “Assess” portion is inhaler technique, adherence and patient preference to ensure that treatment is tailored to the individual patient. Traditionally, asthma has been managed pharmacologically and by avoidance of triggers, with a seemingly “one size fits all” approach.(24)

1.1.5. Management of COPD

The cornerstone of management of stable COPD is risk factor reduction, including smoking cessation. All COPD patients should be encouraged to stop smoking at every opportunity.(27)

Bronchodilator therapy is the main symptomatic therapy for patients with stable COPD. Beneficial effects include reductions in shortness of breath and improved exercise capacity and quality of life.(28) Short-acting beta-agonists (SABAs), mainly salbutamol and terbutaline, are the first-line therapy for symptomatic relief. When disease is no longer controlled with a SABA, a long-acting beta-agonist (LABA) like salmeterol or formoterol or an anti-muscarinic agent like tiotropium should be added.(28) While beta-agonist therapy has been shown to improve exercise capacity, reduce exacerbation rate and improve quality of life, there is no evidence that they prevent lung function decline or improve survival in COPD patients.(29)

Combination therapy with an ICS and LABA (fluticasone/ salmeterol or budesonide/ formoterol) has been shown to reduce exacerbation rates, hospital admissions and all-cause mortality, and to improve lung function.(28) Most patients with moderate to severe COPD are now maintained on a combination ICS/ LABA inhaler.

Other therapeutic options in stable COPD include pulmonary rehabilitation, long term oxygen therapy, immunization and management of extrapulmonary disease.(30)

An exacerbation of COPD is characterized by an acute worsening of a patient's symptoms, which is acute in onset and necessitates a change in regular medication.(31) Management of an acute exacerbation depends on severity. Outpatient management consists of increased bronchodilators, antibiotics and oral corticosteroid therapy.(28) Severe exacerbations are usually managed in the hospital setting with the addition of non-invasive ventilation for symptoms or signs of respiratory failure.(32)

1.1.6. Inhaled medications in asthma and COPD

The following review discusses active drugs, which can be delivered via the Diskus™ DPI, the focus of this thesis. These drugs include salbutamol, salmeterol and fluticasone.

1.1.6.1. Beta-agonists

Inhaled beta-agonists are sympathomimetic agents, which selectively bind to and stimulate beta-2 receptors in the bronchial walls leading to smooth muscle relaxation and bronchodilation.(33) The mechanism of action is via a G-protein coupled second messenger system with subsequent activation of adenylate cyclase and an increase in cyclic AMP (cAMP) levels and protein kinase A (PKA) activity. Bronchial smooth muscle relaxation is mediated via PKA phosphorylation of myosin light chain kinase and calcium-dependent potassium channels.(34)

Effects of beta-2 receptor stimulation include bronchodilation, fine skeletal muscle tremor, skeletal muscle vasodilation, glycogenolysis, myometrial relaxation and mast cell mobilization.(34) Effects of beta-1 receptor stimulation include tachycardia and increased stroke volume, lipolysis, reduced gut motility and secretions and hyper-reninism.(34) The beneficial effects of beta-agonists in respiratory diseases are mediated via beta-2 receptor agonism, whereas the main side effects are mediated via beta-1 receptor agonism.

Inhaled beta-agonists can be divided into short-acting (SABA) and long-acting (LABA) agents based on the half-life of the drug.

Examples of short-acting beta-agonists include salbutamol, levosalbutamol, terbutaline, pirbuterol and metaproterenol. Salbutamol is the most widely used short-acting beta-agonist due to its good safety profile owing to predominant beta-2 receptor binding. It is administered via pMDI, Diskus™ and nebulizer. Salbutamol has an onset of action between 5 and 20 minutes, an elimination half-life of 6.1 ± 2.1 hours and a duration of action of 3 to 6 hours.(34, 35)

Elers et al. showed that median urinary concentrations of salbutamol after inhalation of 0.8 mg of salbutamol ranged from 129.2 to 260.9 ng/ml with the maximal median concentration in samples collected 0-4 hours after administration.(36) Fast absorption was observed after inhaled salbutamol with the time of maximal median serum concentration (T_{max}) being 30-60 minutes and the median serum C_{max} of 1.75 ng/ml and maximum individual serum concentration being 2.74 ng/ml. Other pharmacokinetic studies have shown a T_{max} of 20-25 minutes.(37) Salbutamol is metabolized almost exclusively hepatically, being converted to salbutamol 4'-O-sulfate, which is subsequently excreted in faeces (10%) and urine (90%).(38-40)

The two widely used LABAs are salmeterol and formoterol. Both have a duration of action greater than 12 hours.(41) Salmeterol is usually administered via the Diskus™ DPI or pMDI and formoterol via the Turbuhaler™ DPI.

Plasma salmeterol concentrations of 0.1 to 0.2 and 1 to 2 mcg/L have been attained in healthy volunteers about 5 to 15 minutes after inhalation of a single dose of 50 and 400 mcg, respectively.(42) Salmeterol is available commercially bound to the xinafoate moiety, which has no pharmacologic activity of its own. Both salmeterol and xinafoate are more than 95% protein bound. Salmeterol base is metabolized by cytochrome P_{450} (CYP) 3A4 to alpha-hydroxy salmeterol, which is subsequently eliminated in the faeces (60%) and urine (25%).(42) At the recommended doses of salmeterol, systemic concentrations are low or even undetectable. Although the half-life of the drug is only 5.5 hours, the duration of action is 12 hours and salmeterol has an effective pulmonary half-life of 12 hours based on pharmacodynamic effect and receptor binding kinetics.(43) Based on a population pharmacokinetic study, salmeterol metabolism fits to a two-compartment model with first-order elimination.(44)

A novel group of ultra-long-acting beta-agonists (ultra-LABA) has been marketed recently. These include drugs such as indacaterol, olodaterol, carmoterol, miveterol and vilanterol, which have a duration of action exceeding

20 hours, allowing once daily dosing.(45) Table 1.1 shows properties of a number of ultra-LABAs.

Table 1.1: Pharmacologic properties of ultra-LABA in current clinical use. Reproduced from (45).

Drug	Onset of action (min)	Duration of action (h)	Systemic exposure (% of dose)	Volume of distribution (L)	Protein binding (%)	Metabolism	Elimination
Indacaterol	5	24	44	2361 - 2557	95	Hepatic (CYP 3A4, 2D6, 1A1)	Faeces (>90%)
Vilanterol	5	21.3	27.3	165	93.9	Hepatic (CYP 3A4)	Urine (70%) and faeces (30%)
Olodaterol	5	>24	30	1110	60	Hepatic (CYP 2C9, 2C8, glucuronidation, O-methylation)	Urine and faeces

1.1.6.2. Anticholinergics

Inhaled anticholinergics act by inhibiting muscarinic acetylcholine receptors in the bronchial wall. They can be divided into short-acting agents, such as ipratropium bromide, and long-acting agents, such as tiotropium. Parasympathetic activity is mediated via M1 and M3 muscarinic receptors and results in smooth muscle contraction and mucous secretion.(46) M2 receptors inhibit acetylcholine release from nerve terminals.(47)

Ipratropium inhibits all three muscarinic receptors thus inhibiting the cyclic guanosine 3',5'-monophosphate system at parasympathetic nerve endings. It has a delayed onset of action (45 minutes), elimination half-life of 3.2-3.8 hours and a duration of action of 3-5 hours.(48) Ipratropium has a good side effect profile due to very low systemic concentrations (6.9% systemic bioavailability) and the lack of penetration of the blood-brain barrier.(49)

Tiotropium has higher affinity for the M1 to M3 receptors, but it dissociates rapidly from the M2 receptor and is considered a functionally selective agent.(50, 51) Tiotropium has approximately 2% oral bioavailability and about 20% of an inhaled dose reaches the smaller airways. Clearance is primarily renal.(52) Tiotropium has a prolonged duration of action (functional half-life of 35 hours at the M3 receptor) allowing once daily dosing.(50, 53)

1.1.6.3. Inhaled corticosteroids

Inhaled corticosteroids (ICS) are the mainstay of treatment in COPD and persistent asthma due to their anti-inflammatory effects. ICS reduce airway inflammation and hyper-responsiveness, improve lung function, decrease symptom severity, and reduce the incidence of acute exacerbations.(54) ICS bind to glucocorticoid receptors in the cell cytoplasm; the steroid-receptor complex then translocates to the nucleus, where they regulate the transcription and synthesis of many inflammatory mediators, including cytokines, in eosinophils, monocytes, basophils, lymphocytes and mast cells.(55-57) In patients with persistent asthma, ICS inhibit the late response and bronchial hyper-responsiveness that follows allergen exposure, whereas chronic administration may also reduce the number of mast cells, which may also ameliorate the immediate allergic response.(57)

Systemic exposure to corticosteroids can cause a number of side effects including hypertension, insulin resistance, obesity, easy bruising, poor wound healing, adrenal insufficiency, cataracts, glaucoma, osteopaenia, osteoporosis and suppressed growth velocity in children.(58) Due to the low bioavailability of ICS, inhalation has become the preferred route of delivery in the maintenance therapy of asthma and COPD. Nonetheless, ICS therapy can have local side effects including reflex cough or bronchospasm, dysphonia, oral candidiasis, pharyngitis and sore throat.(59) It is therefore important to monitor for both under-dosing and over-dosing.

The two most commonly prescribed ICS in DPI devices are fluticasone (Diskus^{TM}) and budesonide (Turbuhaler^{TM}). Other available ICS include

beclometasone, ciclesonide, flunisolide, mometasone and triamcinolone.(60) Table 1.2 shows the common pharmacokinetic parameters of these ICS.(59)

Table 1.2: Pharmacokinetic parameters of inhaled corticosteroids. Reproduced from (59).

Drug	Oral bioavailability	Protein-binding (%)	Clearance (L/h)	Volume of distribution (L)	Half-life (h)
Beclometasone dipropionate	15	87	230	20	0.1
Beclometasone monopropionate	26	-	120	424	2.7
Budesonide	11	88	84	183	2.8
Ciclesonide	<1	99	152	207	0.4
Flunisolide	<1	99	396	1190	3.6-5.1
Fluticasone propionate	7	80	58	96	1.6
Mometasone furoate	<1	98	53.5	332	4.5
Triamcinolone acetonide	23	71	37	103	2.0

1.1.7. Inhaler devices

The two main categories of inhaler devices used are pressurized metered dose inhalers (pMDIs) and dry powder inhalers (DPIs). pMDIs utilize a pressurized canister with the drug emitted in a fine spray or mist (see Figure 1.2). Patients must coordinate inhalation with actuation of pMDIs to deliver the maximum dose to the small airways. SABAs like salbutamol are commonly delivered via pMDIs.

Figure 1.2: Components of a pMDI showing canister, plastic sleeve and mouthpiece cap.

DPIs are considered advantageous over pMDIs since they avoid the use of propellants, and are instead actuated during the inhalation.(61) The elimination of propellants allows patient coordination issues to be overcome. DPIs use a dry powder formulation of the drug with an inert carrier such as lactose.(62) Based on the inhaler design and intrinsic resistance, generation of a certain peak inspiratory flow causes de-agglomeration of drug particles to a particle size sufficiently small to reach the smaller airways.(62)

Despite the benefits of DPIs, pMDIs remain very popular. Lavorini et al evaluated retail sales of inhalation devices in European countries and found that average inhaler retail sales (expressed as percentages of total sales) were 47.5% for pMDIs, 39.5% for DPIs and 13% for nebulisers.(63)

Different DPIs require different user techniques for opening, blistering or activation of the dosing chamber and inspiratory flow for optimal drug delivery. The ideal DPI should be effective at reproducibly delivering particles ≤ 6um in size to the patient's smaller airways relatively independent of flow rate, should

be precise in terms of knowing the delivered dose based on the user technique, should have a stable drug formulation, should be user-friendly and should be affordable.(62) Based on the drug half-lives, DPIs also require different dosing frequency, commonly ranging from once daily for newer formulations such as indacaterol/ fluticasone to twice daily for the commonest formulations, salmeterol/ fluticasone and formoterol/ budesonide.(64)

The two most frequently prescribed ICS/ LABA combinations are delivered via the Diskus™ inhaler (for salmeterol/ fluticasone) and the Turbuhaler™ (formoterol/ budesonide).(65) The Turbuhaler™ is an example of a multi-dose reservoir device, which contains a bulk supply of drug from which individual doses are released with each actuation.(66) The Diskus™ inhaler is an example of a multi-unit dose device, which utilizes individually prepared and sealed doses of drug.(66) Tiotropium, used in the treatment of COPD, is usually delivered via the HandiHaler™, which is an example of a single-unit dose device.(66) The new ELLIPTA™ inhaler has been designed to contain two separate blister strips from which inhalation powder can be delivered, and to be simple to use with a large, easy-to-read dose counter.(64) Figure 1.3 shows the design of these four DPI devices. While there are more than 20 other DPI devices currently on the market, these make up a small market share and will not be further discussed.(67) The Seretide or Advair Diskus™ accounts for the largest market share in DPI devices, as evident by IMS Health data in the United States (Figure 1.4).(68) For this reason, the focus of the remainder of this work will be the Diskus™ inhaler.

Figure 1.3: Commonly prescribed inhaler devices: (a) Turbuhaler™ [AstraZeneca], (b) Diskus™ [GlaxoSmithKline], (c) HandiHaler™ [Boehringer Ingelheim], and (d) ELLIPTA™ [GlaxoSmithKline].

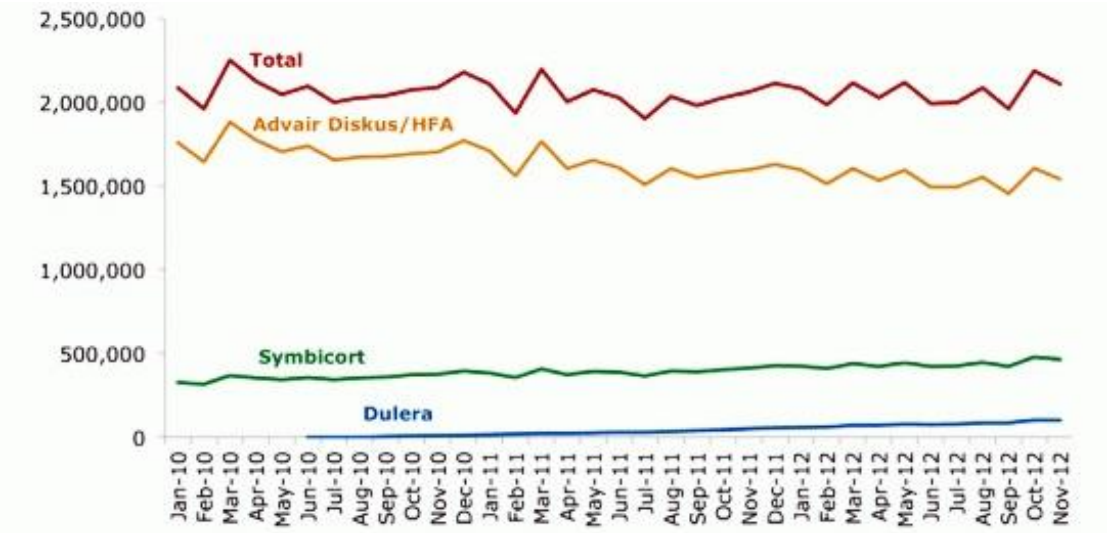

Figure 1.4: Combination inhaler total prescription trends. Reproduced from (68).

1.1.8. Adherence to inhaled therapy

The major benefit of inhaled therapy is direct application of the active drug to the site of action with reduced systemic effects. However, poor adherence to inhaled medications remains one of the main reasons for a suboptimal therapeutic response.

Adherence is defined as the “active, voluntary, and collaborative involvement of the patient in a mutually acceptable course of behavior to produce a therapeutic result”.(69) Non-adherence to inhaled controller medication in asthma and COPD have been recognized as a major problem affecting drug efficacy.

Causes of non-adherence include inadequate health literacy, a lack of understanding about medication efficacy and misunderstanding of directions for use. Poor adherence has also been associated with older age, physical and cognitive function, different types of inhaler devices, design, and technique, patient education and other social factors.(70) There are many different factors that can influence the effectiveness and adherence to medication such as age, physical and cognitive function, different types of inhalers, inhaler design or technique, patient education, provider skills (clinician), society and lifelong therapy.(71) Elderly patients tend to have impairments such as weakness or poor vision and decline in cognitive skills, which make it difficult for them to master the inhaler techniques. Using different types of inhaler with different techniques may lead to incorrect use of the devices. Clinicians should be aware of the guidelines on COPD management to avoid insufficient amount of information received by the patient.(72, 73) Other than that, language barriers and cultural differences could also be an obstacle in explaining the technique of using the device.

Adherence to inhaled medications can be divided into temporal adherence (sometimes simply referred to as adherence) and technique adherence (sometimes referred to as competence). Poor temporal adherence can take the form of underuse, overuse and haphazard use. Technique adherence

assesses proper use of the inhaler device as laid out in the manufacturer's recommendations for use.

1.1.8.1. Methods of assessing inhaler adherence

Adherence to inhaled therapy has traditionally been assessed by using patient questionnaires or diaries, dose counters integrated into DPIs or pMDIs, canister weights in pMDIs, biochemical monitoring or pharmacy registers.(74) Table 1.3 shows the advantages and disadvantages of these traditional methods.

These methods are all very crude measures, which provide an average of adherence usually over a period of 1 to 3 months. Day-to-day or periodic trends in overuse, underuse or haphazard use cannot be objectively measured.

Table 1.3: Advantages and disadvantages of methods for assessing inhaler adherence.

Category	Method	Advantages	Disadvantages
Subjective	Patient self-report questionnaire/ diary	Simple Low cost Questions can be adapted to patient population	Inaccurate Overestimates adherence Patients do not accurately assess their own competence
	Clinician assessment	Simple Low cost Can assess technique and combine outcome measures to assess temporal adherence	Inaccurate Adherence may not correlate with outcome measures in all groups Time-consuming Many clinicians have poor inhaler technique themselves
Objective	Canister weight	Simple Low cost	Incapable of detecting dose dumping or medication sharing Does not assess technique Averages adherence over a period of time
	Pharmacy registers	Simple Moderate cost	Requires proper infrastructure Incapable of detecting dose dumping or medication sharing Averages adherence over a period of time Limits patient choice regarding pharmacy
	Biochemical monitoring	Direct Accurate	Costly Invasive Requires expertise and equipment Depends on half-life of drug Cannot distinguish adherence from technique

1.1.8.3. Methods of assessing inhaler technique

Mechanisms of deposition of aerosol particles can be categorized into inertial impaction at airway bifurcations, sedimentation due to gravity and diffusion due to Brownian motion (Figure 1.5).(75) Recommendations for inhaler technique are devised to minimize deposition in the oropharynx and trachea and maximize deposition in the small airways and alveoli.

Inhaler technique is assessed using standardized checklists and subjective clinician observation, as shown in Table 1.4.(76) Unfortunately, many different checklists exist for individual devices, with 16 in the literature for the Diskus™ device ranging from 3 to 13 steps.(76) Scoring systems also varied with the most common system being assigning a score of 0 for an incorrect step or a score of 1 for a correct step and summing the score from all steps.(77-79)

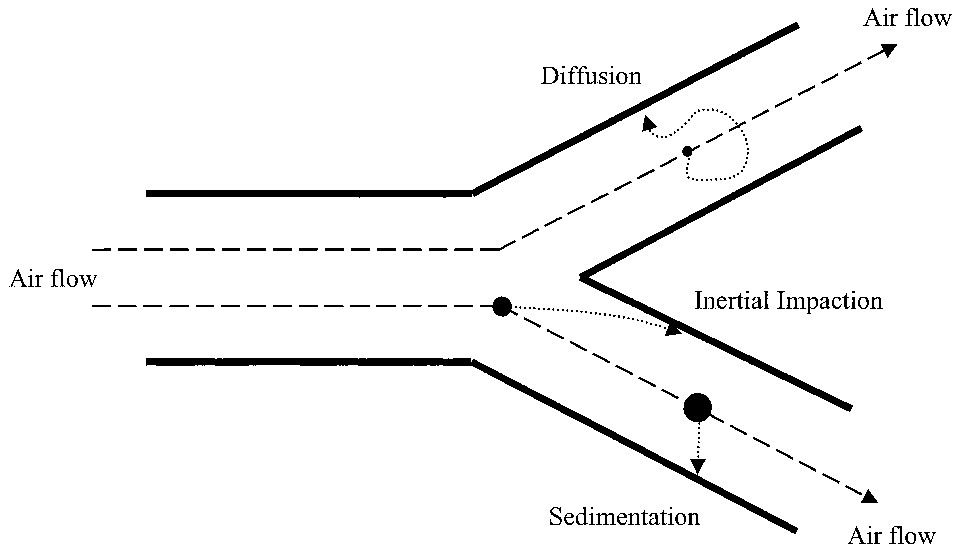

Figure 1.5: Schematic of deposition mechanisms of inertial impaction at airway bifurcations, sedimentation due to gravity, and diffusion due to random Brownian motion. Reproduced from (75).

1.1.9. Diskus™ inhaler technique

The 13 steps for proper use of the Diskus™ inhaler are outlined below.(80)

Table 1.4: Standardized checklist used for evaluation of Diskus™ inhaler technique.

Step	Correct step	Possible technique errors	Is error critical?
1	Push the outer cover as far as possible before the inhalation	Failure to open the outer cover Incomplete opening of outer cover	Yes
2	Slide the lever until the ”click” sound to actuate dose	Failure to slide the lever until the “click” sound	Yes
3	Keep the mouthpiece horizontal or in upward position	Holding the Diskus™ inhaler with the mouthpiece facing downward	Likely significant
4	Exhale into the room and away from the mouthpiece after loading	Exhalation into the device mouthpiece	Likely significant
5	Slowly and completely exhale out to residual volume (to empty the lungs)	(1) No exhalation or insufficient exhalation (2) Forced and fast exhalation	Probable
6	Tilt head back (hyperextend) slightly and keep the device horizontal during inhalation	Lowering one’s head or holding the mouthpiece upward during inhalation	Possible
7	Place teeth over the mouthpiece with lips positioned around it deeply (over tongue) and securely (sealing lip)	(1) Lips surround the mouthpiece shallowly against teeth or tongue (2) Lips are not sealed around the mouthpiece during inhalation	Possible
8	Inhale forcefully from the beginning, slowly (for > 2–3 sec), deeply, uniformly, and continuously inhale during the inspiratory phase until the lungs are full	(1) Gradual increase in the speed of inhalation (2) Fast and extremely forceful inhalation (3) Prematurely stop inhaling (not inhaling to total lung capacity) or inhaling twice or more during the inspiratory phase of the breathing cycle	Likely significant
9	At the end of inhalation remove the inhaler from the mouth and close the lips	Not removing the inhaler from the mouth at the end of inhalation	Unlikely
10	Hold breath for > 5 sec (optimally for 10 sec) after inhalation (an objective measurement performed using a stopwatch)	Not holding breath or holding breath for < 5 sec	Likely significant
11	Exhale slowly through the nose and away from mouthpiece	(1) Breathing out rapidly from the mouth after holding breath (2) Exhaling into the mouthpiece	Possible
12	Recover the lever and the outer cover	Not closing the lever and the outer cover	Unlikely
13	Rinse one’s mouth out after inhaling and do not swallow the rinsing water	(1) Failure to rinse one’s mouth (2) Swallowing the rinsing water	Likely significant for adverse events

The evidence for the significance of errors in each of these steps is reviewed below.

1.1.9.1. Failure to open the outer cover

Failure to open the outer cover of the inhaler means that the dose cannot be actuated and the mouthpiece and air inlet are not accessible for dose inhalation. Zero percent of the drug is available for delivery and this is considered a critical error.

1.1.9.2. Failure to slide the lever until the “click” sound

Failure to slide the lever causes failure to actuate or blister a dose, that is, the dose remains sealed in the blister pack and is not available for delivery. This is a critical error since 0% of the dose is available.

1.1.9.3. Failure to hold the device in the horizontal or up position after dose actuation

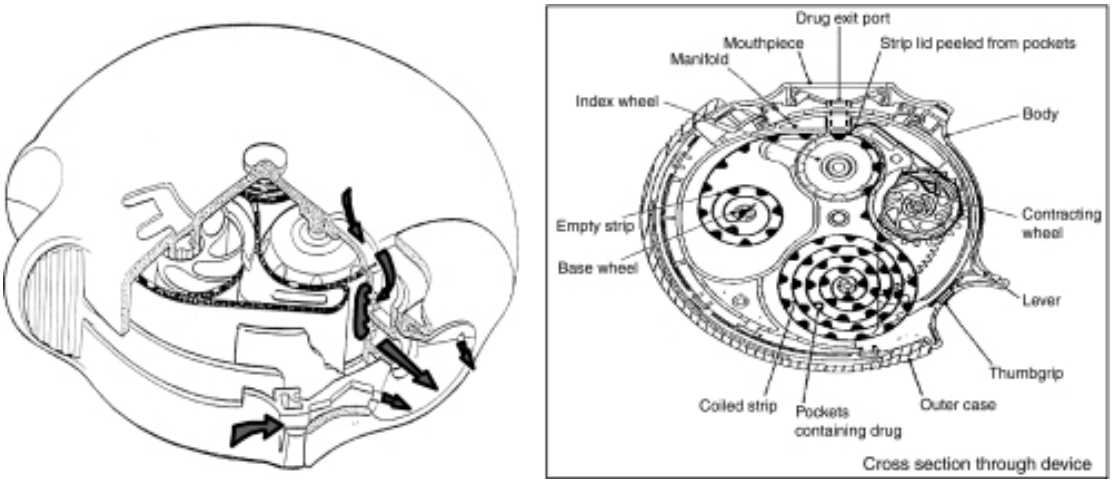

Figure 1.6: Internal components of the Diskus™ inhaler and direction of airflow and air entrainment during inhalation. Reproduced from (66).

Figure 1.6 above shows the major components of the Diskus™ inhaler. The arrows show the direction of airflow during an inhalation. The drug is kept in a pocket in front of the drug exit port and is susceptible to being displaced from this pocket if the inhaler is not held in a horizontal or upward position after the

sealed pocket is opened. Although the manufacturer recommends holding the inhaler in a horizontal or upward position, the effect on drug delivery or available dose of holding the inhaler in other positions has never been formally studied.

1.1.9.4. Exhalation away from the mouthpiece

For DPIs, one frequently observed error is that patients exhale into the device mouthpiece after loading or blistering the dose.(71, 76, 81, 82) Correct DPI inhaler technique involves exhaling away from the inhaler mouthpiece to functional residual capacity or residual volume before the inhalation manoeuvre. Exhaling into a DPI mouthpiece, however, can cause medication to become dispersed or altered, which in turn leads to a reduced quantity of medication available for pulmonary drug delivery. A previous study carried out by Engel et al. first demonstrated this finding using a Turbuhaler™ DPI. They reported that inhalations which were preceded by exhalations into the Turbuhaler™'s mouthpiece resulted in poor bronchodilatation for patients.(83) It was also hypothesized that the introduction of humidity from exhaled air can potentially cause powder to agglomerate, which subsequently reduces the intended deaggregation properties of the inhalation.

It is reported in literature that between 14-22% of patients exhale into their DPI mouthpiece, depending on the type of DPI used.(82) The actual figure may be significantly higher because checklist methods for detecting this error only capture a snapshot of a patient's behaviour at one point in time. The inability of many patients to correctly use their inhaler device may be a direct consequence of insufficient or poor inhaler technique instruction.(71) In a study on pharmacists' knowledge of correct DPI technique, it was reported that the vast majority were unaware of the requirement to exhale away from the device mouthpiece prior to inhalation.(81) Regardless of the causes of this error in technique, the outcomes include a lack of improvement in respiratory symptoms leading to a perceived lack of effectiveness of the medication. This causes clinicians to prescribe higher doses of medication to patients, who may then suffer from adverse reactions and incur higher medication costs.

1.1.9.5. Exhalation to residual volume

A systematic review of inhaler technique found that the most frequent DPI technique error was failure to exhale prior to inhalation (12-77% of errors).(71) Most DPIs require rapid, forceful inhalation to achieve drug de-agglomeration. Manufacturers recommend an exhalation to residual volume or functional residual capacity to facilitate the subsequent deep inspiratory effort required. While many in vivo studies have been done to investigate the impact of exhalation to different lung volumes on pMDI drug delivery,(84) no similar studies exist for DPI devices and the recommendations are based on consensus.

1.1.9.6. Tilt head back (hyperextend) slightly and keep the device horizontal during inhalation

Neck extension is recommended to reduce the angle aerosolized particles must travel to enter the larynx from the oropharynx. Again, it is recommended that the inhaler be held in the horizontal position during inhalation to minimize displacement of the drug from its pocket. There is no data on the in vitro or in vivo effects of this technique error.

1.1.9.7. Place teeth over the mouthpiece with lips positioned around it deeply (over tongue) and securely (sealing lip)

A tight mouthseal is required to ensure that all inspiratory force generated is transferred to the inhaler device. No experimental data exists on the importance of this factor.

1.1.9.8. Inhale forcefully from the beginning, slowly (for > 2–3 sec), deeply, uniformly, and continuously inhale during the inspiratory phase until the lungs are full

For greatest benefit, the maximum amount of drug needs to reach the site of action, that is, the airways. This depends on the patient’s inspiratory flow,

inhaled volume, ramp rate of inhalation and degree of airways obstruction.(85, 86) Findings from previous studies using the Electronic Lung Model showed that in a large subgroup of patients, only 15-30% of the inhaler dose was deposited in the small airways and alveoli of the lung.(10, 66)

For patients using a dry powder inhaler (DPI), de-agglomeration of the active drug from its carrier (typically lactose monohydrate) depends on a combination of factors: turbulence, mechanical impaction, particle uptake and mechanical vibration.(87, 88) One study using a Ventolin Diskhaler™ showed that mechanical impaction was not an effective mechanism for powder de-agglomeration, whereas turbulence was found to have a definite effect.(89) Turbulence leads to aerodynamic lift, drag and shear, as well as separation forces. The turbulent energy generated depends on the intrinsic resistance of the inhaler and the flow rate generated by the patient. Some DPIs have high internal resistance, for example the Turbuhaler™, while some have relatively low resistance, like the Diskus™, as shown in Table 1.5.(90) There is a direct relationship between the intrinsic resistance of a DPI and the peak inspiratory flow rate (PIFR)-dependence for drug delivery (Figure 1.7). There is some in vitro evidence that the Diskus™ device is less effort-dependent than other high resistance inhalers (91); however, there is consensus that PIFR is an important factor for all current DPIs. Regardless, it is recommended that optimal drug delivery is achieved with a flow rate of greater than 30 l/min and ideally, greater than 60 l/min.(92)

For traditional DPIs, insufficient PIFR can lead to ineffective drug delivery resulting in unintentional non-adherence and poor clinical outcomes. Conversely, some authors have advised that very high inhalation flow rates can lead to increased throat deposition and exhalation of particles that are less than 1 µm in aerodynamic particle size.(93, 94) While modern, sophistically engineered powders and inhaler devices are less flow-rate dependent, or even flow-rate independent (95), it is our experience that the majority of patients with obstructive airways disease are currently prescribed traditional DPIs like the Seretide Diskus™ or Symbicort Turbuhaler™.

Table 1.5: Internal resistance of common dry powder inhaler devices. Adapted from (91). Resistance is given by the ratio of the square root of pressure drop to airflow rate. A 'medium' resistance (0.033 $kPa^{0.5}$.min/L) inhaler device requires a pressure drop of 4 kPa to draw an airflow rate of 60 L/min through it.

Inhaler	Resistance ($kPa^{0.5}$.min/L)
Diskhaler (GlaxoSmithKline)	0.032
Diskus (GlaxoSmithKline)	0.034
Handihaler (Boehringer Ingelheim Pharmaceuticals)	0.042
Inhalator (Boehringer Ingelheim Pharmaceuticals)	0.051 – 0.062
ISF inhaler (Cyclohaler, Pharmachemie)	0.019
Rotahaler (GlaxoSmithKline)	0.015
Ratiopharm Jethaler (Ratiopharm)	0.036
Spinhaler (Aventis)	0.016
Turbuhaler (AstraZeneca)	0.043

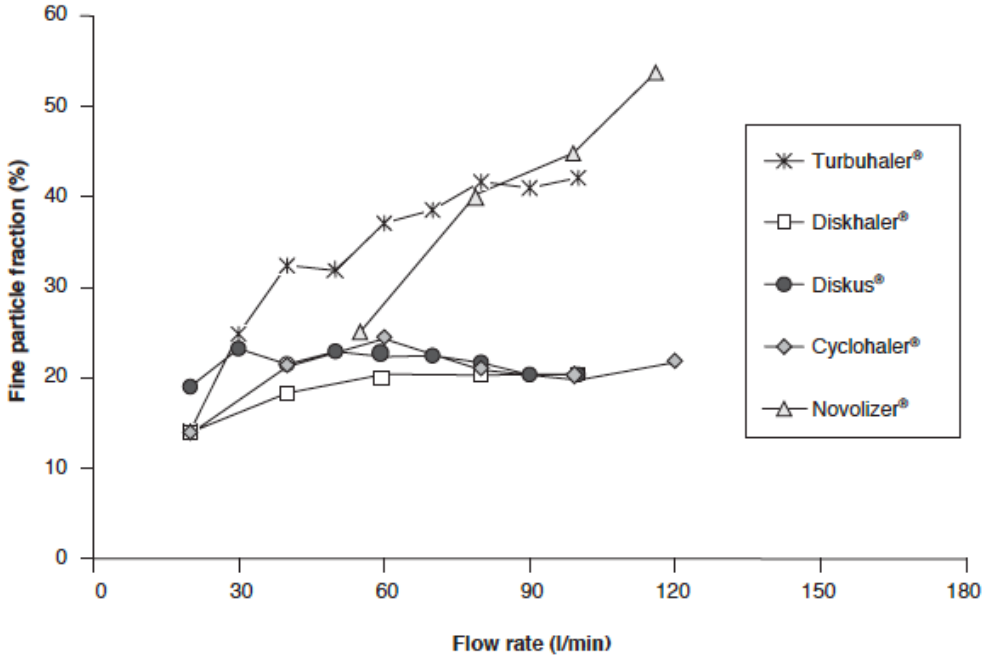

Figure 1.7: Relationship between peak inspiratory flow rate and fine particle fraction emitted for five common dry powder inhalers. Reproduced from (91).

1.1.9.9. At the end of inhalation remove the inhaler from the mouth and close the lips

Closing the lips after exhalation is related to the next step of breath holding.

1.1.9.10. Hold breath for > 5 sec (optimally for 10 sec) after inhalation (an objective measurement performed using a stopwatch)

The effect of breath holding duration on drug delivery has been well-studied in pMDI devices. Breath holding after inhalation enables particles delivered to the smaller airways to be deposited in that region, instead of being exhaled.(96) The breath hold increases particle residence time and increasing the breath hold duration from 0 seconds to 10 seconds leads to an 8-fold increase in particle (< 1um) deposition in the smaller airways.(91) An in silico study of aerosol deposition using the Electronic Lung Model showed that breath hold duration has minimal effect on tracheobronchial deposition but is positively correlated with pulmonary deposition.(86) Similar in silico studies using aerosolized insulin have shown that breath holding allows the mechanisms of sedimentation and diffusion more time to act, thus increasing particle deposition in the smaller airways, as shown in Figure 1.8.(75)

1.1.9.11. Exhale slowly through the nose and away from mouthpiece

This step prevents humidified air from entering the device and is likely less important for a multi-unit dose device like the Diskus™ inhaler compared to bulk storage devices like the Turbuhaler™. There is no data on the effect of forceful exhalation or exhalation through the mouth on subsequent drug efficacy. These effects may be more dependent on the duration of the prior breath hold.

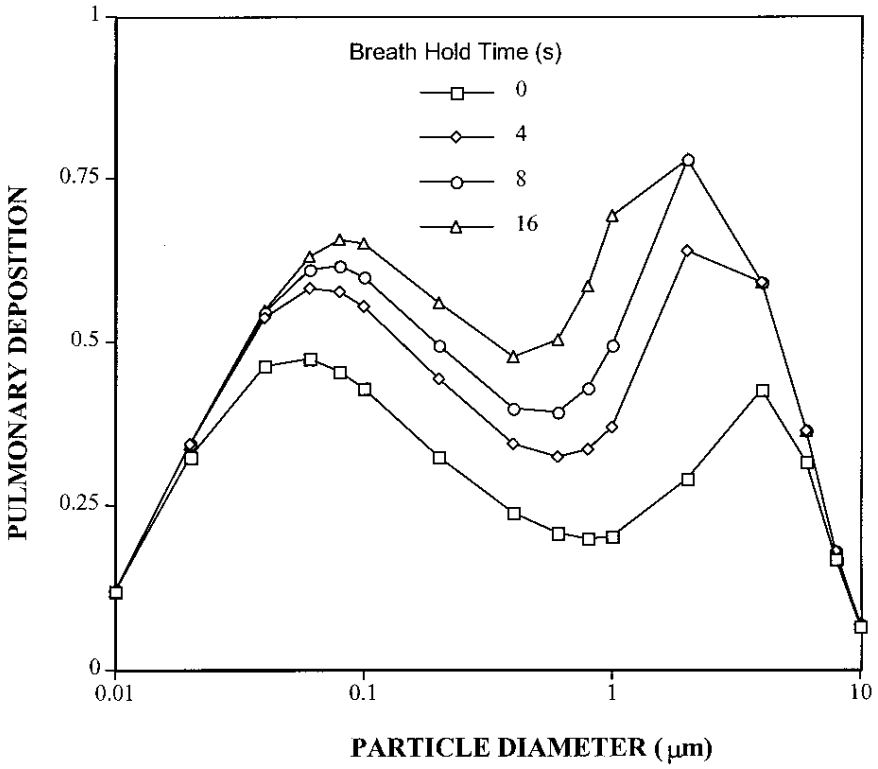

Figure 1.8: Relationship between breath hold duration, particle diameter and pulmonary deposition. Higher breath hold durations are associated with increased pulmonary deposition. Reproduced from (75).

1.1.9.12. Recover the lever and the outer cover

This step relates to device storage. Recovering the outer cover automatically resets the dosing lever, making the device ready for the next actuation. It also closes the drug exit port, reducing exposure of the internal components to the elements. Again, with a multi-unit dose device where all doses are contained in sealed pockets, the effect of not recovering the outer cover is likely insignificant but has never been formally studied.

1.1.9.13. Rinse one's mouth out after inhaling and do not swallow the rinsing water

Mouth rinsing is important with steroid inhalers to reduce the risk of oral candidiasis. This step will not be discussed further since it is related to adverse effects and not to drug delivery or efficacy.

1.1.10. Estimates of temporal and technique adherence

Feehan et al. studied the dispensing records of 2193 patients who received controller medications for asthma in a 12-month period and found that only 14-16% of patients had 'satisfactory' adherence (adherence ≥ 80%).(97) Numerous other studies have found that adherence to inhaled controller medications is generally poor with temporal adherence estimates ranging from 14 to 40%.(98, 99) Non-adherence to controller therapy leads to poor asthma control, higher rates of exacerbations and increased healthcare utilization in both adult and paediatric populations.(100) Compared to other chronic diseases, patients with COPD have a lower mean adherence rate of 68.8% to medical treatments.(101) In a study of adherence of COPD patients with inhaler therapy for 5 years, it was found that inhaler compliance had declined over the 5 year period from more than 60% to less than 50%.(102) Poor adherence in COPD has also been associated with increased hospital admissions, length of hospital stay and reduced quality of life.(103)

While the majority of adherence studies in chronic diseases assess mainly temporal adherence, several studies have highlighted that errors in inhaler technique may be as detrimental as the lack of temporal adherence.(104-106) In 2000, Cochrane et al. summarized all papers describing inhaler technique and concluded that the frequency of efficient inhalation technique ranged from 46–59%.(107) Hesselink et al. showed that about 24% of patients using DPIs made at least one critical error causing detrimental effects on drug delivery and efficacy.(108) Table 1.6 shows the breakdown of technique errors in Diskus™ inhaler use in the published literature. The most common technique errors when using the Diskus™ inhaler are errors in exhalation prior to inhalation, breath holding, incorrect mouthpiece positioning and incorrect dose metering. However, the study by Li et al. in a Chinese population found that errors in inhalation were most common (109).

Table 1.6: Breakdown of technique errors in Diskus™ inhaler use in the published literature. For each considered study, values represent the percentage of patients showing specific errors in the use of the dry powder inhaler (DPI).

Study	Incorrect dose metering	Incorrect inhaler positioning	No exhalation before activation	Incorrect mouthpiece positioning	No forceful and deep inhalation	No breath hold	Failure to breathe out slowly
Molimard et al. (110) (n=3811)	-	-	30	-	-	26	-
Van der Palen et al. (111) (n=50)	8	-	40	-	-	6	2
Girodet et al. (112) (n=984)	-	-	40	9	-	36	-
De Angelis et al. (113) (n=358)	-	-	18	-	-	-	-
Li et al. (109) (n=384)	30	25	48	65	94	90	23

1.1.11. Improved methods for monitoring adherence and technique

Electronic monitors, developed over the last two decades, have become the gold standard for measuring adherence.(114) These methods are objective and allow more accurate comparison of changes in adherence from a patient's baseline. The majority of electronic monitors were developed for the pMDI and monitor temporal adherence only.

The Smartinhaler and Doser CT devices record the date, time and number of actuations of a pMDI using a pressure sensor, which detects actuations. (115-117) The SmartTrak inhaler is a newer monitoring device for the pMDI, which allows remote upload and ringtone reminder capabilities and generates

a graphical display of medication use for patient- and physician-feedback.(118) The Spiroscout system uses Global Positioning System (GPS) technology to track both when and where a patient uses his/ her inhaler.(74) Newer devices, such as the AdHaler and I-neb Adaptive Aerosol Delivery System utilize mobile telecommunications networks to transmit data wirelessly to clinicians or other healthcare professionals who can provide feedback to the patient.(74) The Diskus™ Adherence Logger was developed specifically for the Diskus™ DPI; a magnetic sensor is used to detect motion of the drug delivery lever.(119) The device also features software, which allows the adherence data to be uploaded to a computer for display and analysis.

Although some of the above devices allow a graphical display of both date and time of inhaler use, most studies using electronic recording devices have reported adherence as the mean daily dose, or mean dose over the study period.(120-122) There are limitations to this method; for example, the mean rate of adherence is the same whether an individual took the medication according to the prescribed schedule or one who took all the doses in the first half of a dosing period, leaving none in the second half.

Inhaled medications add a further challenge because electronic recording devices usually do not assess if the inhaler was taken correctly.(71, 76, 82, 123-126) An individual may take their inhaler according to the dosing schedule but with incorrect technique leading to minimal or no clinical benefit. In this case the average use over time is meaningless unless data on the technique of use is also incorporated into the calculation of adherence. Hence, there is a need to develop a method to quantify adherence that accounts for variations in dosing schedules and inhaler user technique as these features influence the pharmacokinetic profile of the medication.

There have been increased attempts recently to develop electronic monitors for both temporal and technique adherence. The MDILog monitors the shaking of the pMDI canister and the timing of actuation.(127) The SmartMist microprocessor-assisted system, now discontinued, analyses the inspiratory flow profile and automatically actuates the MDI when predefined conditions of

flow rate and cumulative inspired volume coincide.(128) There has also been a push towards tele-health monitoring systems, such as the I-neb Insight Online, where adherence data is pushed to a secure server, which can be accessed by patients and clinicians.(129) A competence monitor for the Diskus™, called the DPILog, has also been developed but it does not assess all aspects in Diskus™ technique.

Apart from longitudinal monitors, two technologies allow estimation of the peak inspiratory flow rate generated by a patient when an inhaler's intrinsic resistance is simulated. One such device is the Clement-Clarke In-Check Dial, which uses apertures of different sizes to simulate an inhaler's resistance to airflow. The device can be used to measure the inhalation rate of a patient when they use each of the commonly prescribed inhalers that are currently available and also to select an appropriate inhaler.(130) An accuracy study showed that there was a constant bias of 3.9 l/min for the Diskus™ and 3.5 l/min for the Turbuhaler™ when the In-Check Dial was compared to an inhaler profile recorder.(131) Mahler et al. showed that approximately 20% of COPD patients greater than 60 years of age had a subotimal PIFR, when measured using the In-Check Dial.(132) Melani and colleagues reported that 24% of Turbuhaler™ users had suboptimal PIFR (< 60 l/min), of which 77% had a PIFR < 30 l/min, and 12% of Diskus™ users had suboptimal PIFR, of which 60% had a PIFR < 30 l/min.(133) The downside of using the In-Check Dial for longitudinal monitoring relates to the fact that patients must use a separate device and there may be natural variation in the PIFR achieved with the DPI and with the In-Check Dial even when used consecutively within a short period of time.

The Vitalograph Aerosol Inhalation Monitor uses a hygienic single-use disposable inhaler simulator to provide information regarding inspiratory acceleration at start of inspiration, timing of firing of MDI / activation of DPI, inspiratory flow rate throughout inspiration, inhalation time within target flow range and breath hold time at end of inhalation.(134)

1.1.12. Impact of feedback and training on adherence

There is overwhelming evidence that feedback and training improve both temporal and technique adherence. Jolly et al. studied the effect of educational training on MDI technique and found that the median inhaler proficiency score (based on the number of correct steps executed) increased from 3 to 6 after one education session and to 8 after three sessions. Scores decreased one month after training and again increased with further training.(135) In one randomized controlled trial, MDI technique training lead to improvements of proficiency scores from 6.2 to 10.7.(136) Another RCT in hospitalized patients found that 62% and 78% misused their MDI and Diskus™ inhalers, respectively.(137)

Electronic monitors also assist in improving adherence. Nides et al. showed that participants that received feedback based on an electronic monitor at the 4-month follow-up stage adhered more closely to the prescribed three sets per day (mean 1.95 versus 1.65) and used the prescribed two actuations in a greater percentage of sets (80% versus 60.3%).(138) Another study investigated whether direct clinician-to-patient feedback discussion on their inhaled steroid and beta-agonist use on all visits influenced adherence and reported that adherence increased form 61% to 81% by week two and remained above 71% for the entire study compared to the control group, for which adherence steadily decreased from 51%.(139) Charles et al. also showed that an audiovisual reminder device lead to a 26% higher adherence rate in the treatment group compared to the control group.(140)

1.2. Rationale

There is a need to develop a comprehensive system for monitoring patient inhaler temporal and technique adherence in order to tailor and personalize training and feedback. While current electronic monitors are able to accurately log the date and time of inhaler use, they have fallen short of capturing all aspects of inhaler technique. The majority of monitors have also been produced for the MDI inhaler and there is a clinical need for advanced DPI monitors.

Although checklists are the current “gold standard” for assessing inhaler technique, there is also a lack of data in the literature and general consensus on the significance of all of the errors assessed by these checklists.

This thesis builds on the work done by a team of biomedical engineers and physicians in conjunction with Vitalograph [Ennis, Ireland] to develop an acoustic method of monitoring temporal and technique adherence using the Diskus^{TM} DPI, the most commonly used DPI device.

1.3. The Inhaler Compliance Assessment (INCA™) device

The INCA™ device consists of a microphone, a battery, a memory card and a microprocessor for recording audio and carrying out other processes required for its operation (Figure 1.9). The main criteria for each of these components are small physical size and low power battery consumption in order that the INCA™ device is small enough to be attached to an inhaler and so that it can record for at least one month. The prototype device was attached to a Diskus™ inhaler.

To minimise battery use and to record the time of inhaler use, recording was initiated by the rotation of the inhaler, this is the initial stage in the use of a Diskus™ inhaler. The recording finishes when the device is closed by the user. Each time a recording begins the time from an electronic real time clock is stored as part of the recording's metadata. When the inhaler is returned to the clinic, the data is downloaded onto a desktop PC for analysis. The audio is recorded at a sampling rate of 8 kHz with an 8 bit sampling resolution. The package instructions accompanying a Diskus™ inhaler describes the steps required for its correct use. Six critical phases were identified by acoustic analysis; opening the device, exhalation, drug release, inhalation, >5 second pause of breath holding and exhalation. Figure 1.10 demonstrates how each of these phases can be identified visually from a display of an acoustic recording created by the INCA™ device.

The steps in inhaler use have distinct acoustic characteristics, these characteristics can be used to identify technique errors in inhaler use. For example, the lever movement and blistering of the drug during priming is characterized by a short burst of energy lasting approximately 20-30 ms with a high frequency content (~2 kHz) preceded by a short burst of lower frequency noise (~1 kHz). An exhalation has a sharp increase in amplitude that tapers off with time and the power of exhalation decreases exponentially from 2 kHz to 500 Hz while the spectral power for inhalations are higher and they have a low increase in amplitude compared to that of exhalations, which is maintained for an average duration of 1.8 seconds. These characteristics enabled

identification of each step required for drug delivery. Visual and aural analysis was carried out using a commercially available audio processing software package Audacity®.

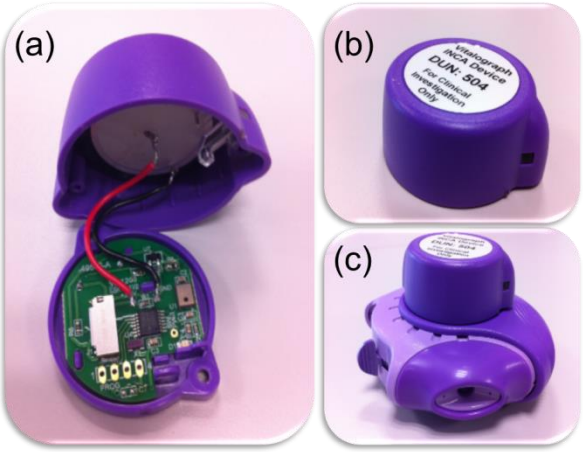

Figure 1.9: INCA™ -enabled Diskus™ inhaler showing profile and internal components of the device.

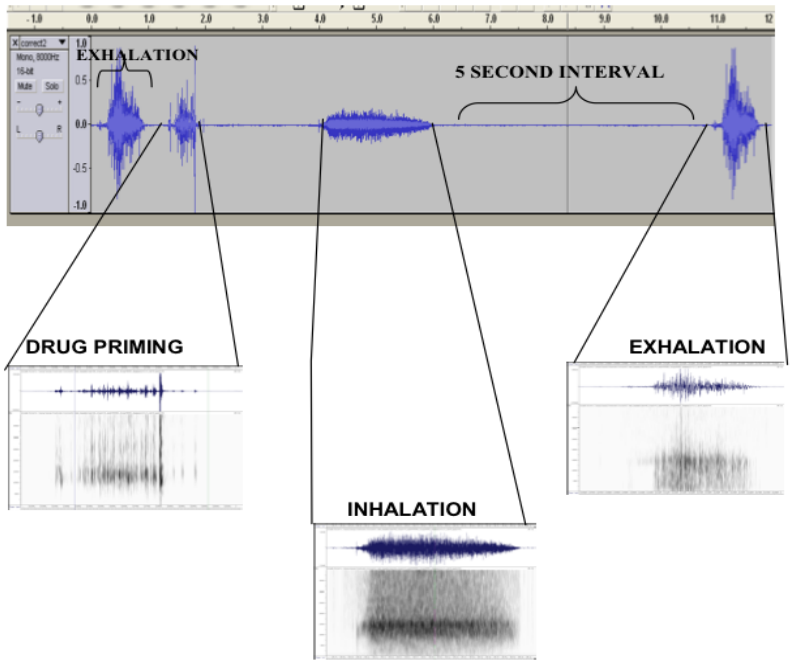

Figure 1.10: A visual display of correct inhaler use: important steps in correct inhaler use are presented in both the time and frequency domain.

1.4. Aims and objectives

1.4.1. Aim 1 (Chapter 3)

- To determine temporal adherence rates, the patterns of adherence and the frequency of Diskus™ DPI technique errors in a community care setting.

1.4.2. Aim 2 (Chapter 4)

- To determine the relationship between underlying disease, age, gender and baseline spirometric parameters on the peak inspiratory flow rate and inspiratory volume from a Diskus™ DPI.
- To measure the effect of the peak inspiratory flow rate and inspiratory volume on the delivered dose from a Diskus™ DPI.
- To develop and validate an acoustic method of estimating the peak inspiratory flow rate and inspiratory volume from a Diskus™ DPI.

1.4.3. Aim 3 (Chapter 5)

- To assess the effect of exhalation into the Diskus™ DPI after blistering on the dose available for subsequent delivery.
- To develop and validate an acoustic method of estimating the direction, distance and flow rate of an exhalation directed at the Diskus™ DPI.

1.4.4. Aim 4 (Chapter 6)

- To determine the effect of spatial orientation of the Diskus™ DPI after dose blistering on the available dose for subsequent delivery.

1.4.5. Aim 5 (Chapter 7)

- To investigate whether the duration of the breath hold after dose inhalation affects the delivered dose from the Diskus™ DPI.

1.4.6. Aim 6 (Chapter 8)

- To assess the contribution of missed doses to pharmacokinetic trough and peak levels achieved after repeated dosing.

1.4.7. Aim 7 (Chapter 9)

- To develop an algorithm for combining data on temporal adherence and technique errors derived from the INCA™ device into a single “actual adherence” metric.
- To compare dose counter, INCA™ dose and actual adherence rates in a cohort of asthmatic patients monitored longitudinally.

CHAPTER 2 - Methods

2.1. How common are Diskus™ inhaler temporal and technique errors in a community care setting?

2.1.1. INCA™ device

The recording device selected to obtain the acoustic signals of inhaler use was the INCA™ (Inhaler Compliance Assessment) device, described above.

2.1.2. Study design

This was a prospective observational cohort study. Patients were recruited from six general practice (GP) clinics and twelve community pharmacies based in Ireland. Over a 2-4 week period, consecutive patients with a history of respiratory illness and already prescribed a salmeterol/fluticasone Diskus™ inhaler were asked to participate. Patients gave informed consent to participate in this study of adherence. Both clinicians and patients were fully aware that the device was an acoustic recording device and that both time of use and inhaler technique were being assessed. Once consented, patients were given an INCA™ enabled inhaler for 1 month and were asked to use it as they normally would and return it at the end of one month.

Demographic data on age, sex, clinical diagnosis, smoking history, education level, socio-economic class, number of exacerbations, hospital admissions and GP/healthcare use in the last year were also recorded.

2.1.2. Ethical and consent considerations

This study was approved by the ethics committee of the Irish College of General Practitioners and the Royal College of Surgeons in Ireland (NCT02552472). Unless participants specifically requested to be shown how to use their inhaler, inhaler technique training was not performed, since the purpose of the study was to assess inhaler adherence in a real world setting.

2.1.3. INCA™ data processing

Each audio file, representing each time the inhaler was used, was assessed by two separate trained raters who used a commercial software analysis program, Audacity® version 2.04 [http://audacity.sourceforge.net/], to visualize and listen to inhaler sounds in order to classify inhaler events. Agreement between the two raters was 83% and disagreements were reconciled by consensus agreement. Most of the differences observed between the raters were due to the classification decision of poor inspiratory flow.

2.1.4. Statistical analysis

Data was analyzed using Stata version 13 [Statacorp, TX, USA]. Descriptive statistics were used to describe the patient characteristics and the errors in inhaler use. The INCA™ adherence rates were calculated as the area under the curve (AUC), using the trapezoid formula:

$$\int_a^b f(x) \approx (b-a)\left[\frac{f(a)+f(b)}{2}\right]$$

Where $f(a)$ is the length of the first wall of the trapezoid and $f(b)$ is the length of the second wall of the trapezoid and $(b-a)$is the width of the trapezoid. The adherence rates calculated included the attempted rate (how frequently the participant tried to take their medication, i.e. evidence of drug priming in audio analysis), the technique rate (how frequently the participant made critical technique errors) and the actual rate (incorporating time of use, interval between doses and critical technique errors). A negative binomial regression model was used to determine trends in technique errors. The number of attempted doses was used as the offset term and age, gender, smoking history, education (primary/secondary), GP use, diagnosis, socio-economic class, exacerbations and hospitalizations were included as fixed effects in the model. An ordinary linear regression model was used to determine trends in the actual adherence rate and technique errors to identify possible correlations with demographic and clinical features of the patients. P-values <0.05 were deemed statistically significant.

2.2. The effect of inhalation parameters on delivered dose and an acoustic method to quantify this effect

2.2.1. The relationship between baseline spirometric PIFR and Diskus™ inhalation parameters: Is baseline spirometry sufficient for estimating peak flow from a Diskus™ DPI?

2.2.1.1. Participants

Eighty-five subjects older than 18 years of age from a population of healthy volunteers and patients with asthma, COPD, neuromuscular disease and non-respiratory disorders were recruited by clustered and stratified sampling. Patients were recruited from different clinics in Beaumont Hospital in Dublin, Ireland. There were no specific exclusion criteria for this study apart from capacity to comply with instructions. Informed consent was obtained for the study with explanations of the study protocol.

2.2.1.2. Ethics

This study was approved by the local Hospital Ethics Committee (ERC/ IRB 13/36) and was performed in accordance with the ethical standards laid down in the 2000 Declaration of Helsinki.(141)

2.2.1.3. Flow experimental design

Flow and volume readings were taken while patients used a Diskus™ DPI. Several studies have previously employed an airtight container to connect an inhaler to a spirometer in order to obtain flow measurements through an inhaler device.(142, 143) The construction of the airtight container with the Diskus™ inhaler, spirometer connection and Fleish Pneumotachograph 6800 spirometer used in this study are shown in Figure 2.1.

The spirometer used was the Vitalograph Pneumotrac (Model 6800) [Vitalograph, Ennis, Ireland]. This spirometer uses a Fleisch

pneumotachograph for flow/volume readings. The specifications are presented below:

- Flow Detection Principle: Fleisch type pneumotachograph
- Volume Detection: Flow integration sampling @ 100 Hz
- Accuracy when in Operating Range:
  - Volumes: Better than ± 3% (Max 8 L / Min 0.05 L)
  - Flows: Better than ± 5% (Max 16 L/s / Min 0.02 L/s)
  - Linearity: ± 1% in range 0.1 L/s to 16 L/s
- Resistance: <1.2 $cmH_2O.s/L$ at 14 L/s
- Performance Standards: ISO 26782:2009, ISO 23747:2007, ATS/ERS 2005

Briefly, the airtight container ensured that all inspired air through the mouthpiece of the inhaler comes through the spirometer where it can be measured. In this study a clear PET (Polyethylene Terephthalate) container was used to act as an airtight adaptor between a Diskus™ inhaler and a spirometer. An empty Diskus™ inhaler was placed into the container, which had a custom aperture cut for the mouthpiece and the spirometer connector. The mouthpiece was extended out 1cm in length in order for subjects to get a good seal around the mouthpiece. Steinel Hybond 86 adhesive was used to seal any gaps and prevent any unintentional air from going in or out of the container. The container was submerged in a water bath before each test in order to verify that it was airtight. The end result was that air could only enter or exit via the inhaler mouthpiece and through the spirometer connector.

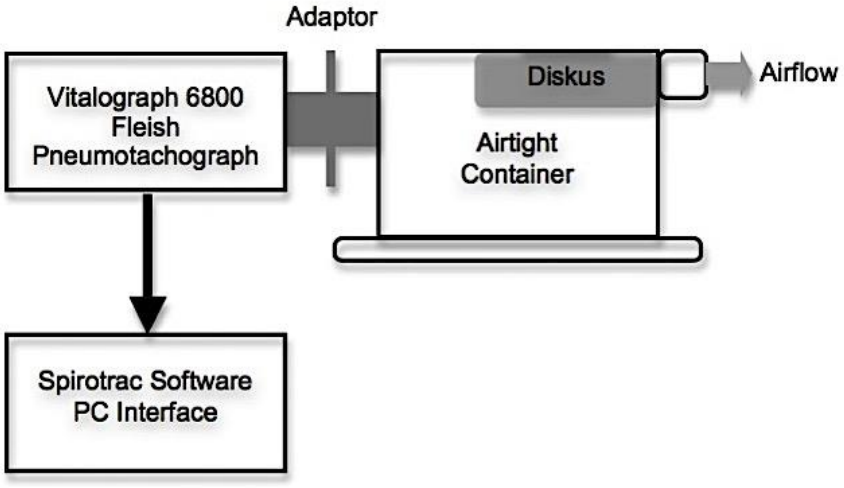

Figure 2.1: Experimental setup showing Diskus™ inhaler in airtight container with adaptor connector to Fleisch pneumotachograph spirometer and PC. The arrow indicates direction of airflow during inhalation.

2.2.1.4. Study protocol

Demographics and baseline lung function by spirometry were recorded. Baseline lung function was taken as the best of three trials. Documented parameters included forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), FEV1/FVC, forced inspiratory vital capacity (FIVC) and peak inspiratory flow rate (PIFR).

Patients were instructed to exhale gently to functional residual capacity and then inhale at maximal flow rate and duration. Each patient performed this manoeuvre until two consecutive PIFR readings were within 20% of each other. Values for Inspiratory vital capacity and peak inspiratory flow rate from the Diskus™ were obtained.

2.2.1.5. Data analysis

Statistical analysis was done using Audacity version 2.0.5., MATLAB _R2013b [Mathworks, Cambridge, UK] and Stata SE version 12. Ordinary least squares regression of spirometric PIFR versus Diskus^{TM} PIFR was performed to determine the degree of correlation between baseline spirometry and flow or volume inhaled while using the Diskus^{TM} DPI. A stepwise deletion linear regression was also performed to determine the relationship between Diskus^{TM} PIFR and the independent variables: condition (categorical), age, gender (categorical), height, weight, BMI, FEV_1, FVC, FEV_1/FVC, spirometric PIFR and spirometric IC with a significance level for removal from the model of 0.05.

Subjects were classified into subgroups of healthy/ non-respiratory condition (NRC), asthma, COPD/ alpha-1-antitrypsin deficiency, and neuromuscular disease (NMD). Subgroup analyses were performed for baseline spirometry and Diskus^{TM} spirometry. Multiple t-tests were done to compare the means for spirometric PIFR, Diskus^{TM} PIFR, spirometric IC and Diskus^{TM} IC for each group. The proportion of patients in each category with a flow rate < 60 l/min and/ or an inspiratory volume < 1 L was also compared. Patients of age greater than or equal to fifty years old and less than fifty years were also compared in the same way.

2.2.2. Developing an acoustic method of estimating inspiratory flow rate and volume from an inhaler

2.2.2.1. Participants

Fifteen healthy volunteers between the ages of 18-40 years were recruited. Subjects were excluded if they had any cardiac, respiratory, hepatic, renal dysfunction, recent respiratory tract infection in the last six weeks, a greater than ten pack/year smoking history, a history of drug/alcohol abuse or a known sensitivity to salmeterol or fluticasone. Baseline spirometry was performed according to ATS recommendations to confirm that subjects had normal lung function.(144) This study was approved by the local Hospital Ethics Committee (ERC/ IRB 13/36).

2.2.2.2. Acoustic Recording Device

The INCA^{TM} device was used to capture an acoustic profile of inhalation while a subject used the Diskus^{TM} inhaler. Acoustic signal processing is outlined below.

2.2.2.3. Flow Experimental Design

The apparatus used in section 2.2.1.3. above was used to measure flow rates and volumes from the Diskus^{TM} inhaler. The INCA^{TM} recording device was attached to the top of the Diskus^{TM}, as shown in Figures 2.2 and 2.3 below.

Figure 2.2. Airtight container with Diskus™ inhaler placed inside and INCA™ device attached on top used to measure the acoustic profile, the flow rate and the volume of an inhalation.

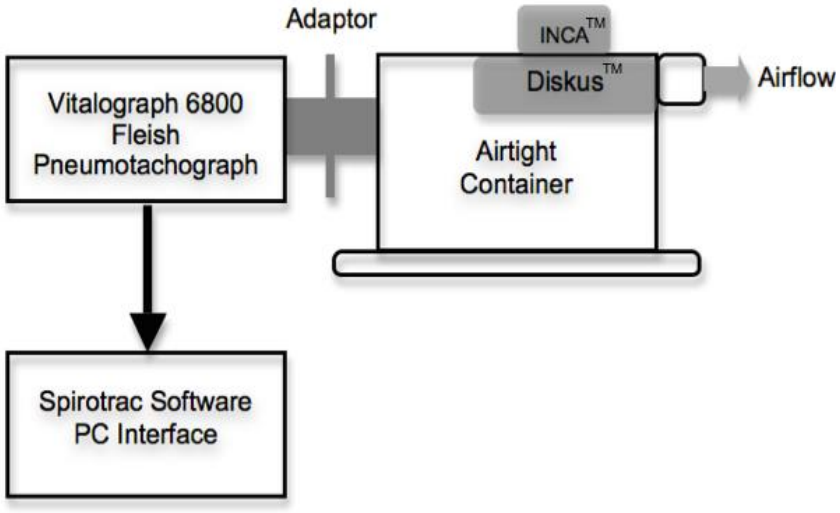

Figure 2.3. Apparatus used for obtaining flow-volume recordings and acoustic measurements from an inhalation maneuver.

2.2.2.4. Test Procedure

The airtight container was connected to the spirometer. Patients were instructed to exhale gently (to functional residual capacity) and then inhale at a variety of flow rates and volumes. Each patient performed this manoeuvre six to eight separate times. The airtight container was sterilized with 100% ethanol and tested for air leaks between subjects. A graphical representation of the overall test set up can be seen in Figure 2.3.

2.2.2.5. Inhalation Signal Analysis

The inhalation audio signals were divided into 1,024 data samples with 50% overlap between successive segments. A Hanning window was used to analyze each segment, while a fast fourier transform (FFT) was used to calculate the power spectral density. Three measures of amplitude were employed in this study; median amplitude (MA), mean absolute deviation (MAD) of the amplitude and root mean square (RMS) of the amplitude. These three measures of amplitude were chosen in order to investigate which had the best correlation with PIFR and IC.

MA was computed using a relative peak detection method. Peaks of the inhalation signal were selected that were greater than their nearest neighbor by a minimum threshold height difference of 200. MAD is the mean of the absolute deviations from the central value. This measure addresses the problem of calculating the mean from a sinusoidal measure and was calculated using the following equation (2.1):

$$MAD = \frac{1}{n} \sum_{i=1}^{n} |x_i - \overline{x}| \qquad (2.1)$$

The RMS or quadratic mean is a statistical measure of the effective value of a signal's amplitude, including the mean value. It takes into account sinusoidal waveforms and gives the equivalent non-varying power of a varying waveform. It is the square root of the mean of the squares of the values of either a

discrete or continuously varying function. RMS has been used in a previous study, which investigated the volume-dependent changes in regional lung sound amplitudes.(145) It was calculated using the following equation (2.2):

$$RMS = \left[\frac{1}{n}\left(x_1^2 + x_2^2 + x_3^2 + \cdots + x_n^2\right)\right]^{\frac{1}{2}} \quad (2.2)$$

The average power (P_{ave}) of each inhalation was calculated in the frequency bands: 20-40 Hz, 40-70 Hz, 70-150 Hz, 150-300 Hz, and 300-600 Hz, in addition to 70-300 Hz, 70-450 Hz, 100-300 Hz, 100-450 Hz and 150-450 Hz. These frequency bands were chosen as they were previously used in a study by Hossain and Moussavi, which investigated the best frequency band to estimate flow rate from respiratory sounds obtained from the chest wall.(146)

In spirometry, the area under a PIFR – time curve equates to the volume of an inhalation or IC. Since acoustic measurements were used to predict the PIFR, integration could not be used to determine IC. Instead it was noted that the area under the curve of the inhalational sound waveform (inhalation volume) approximates that of the area of a semi-ellipse (Figure 2.4), described by the following equation (2.3):

$$Inhalation\ Volume = \frac{1}{2} * pi * \frac{A*B}{2}, \quad (2.3)$$
$$where\ A = PIFR\ and\ B = duration$$

Values for MA, MAD, RMS and P_{ave} for each inhalation were employed to obtain predicted values for the mean PIFR. These predicted mean PIFR values and the actual duration of the inhalation were used to calculate a predicted IC value (Equation 2.3). The predicted values for IC were then compared to the actual IC values for each inhalation, as obtained from the spirometer.

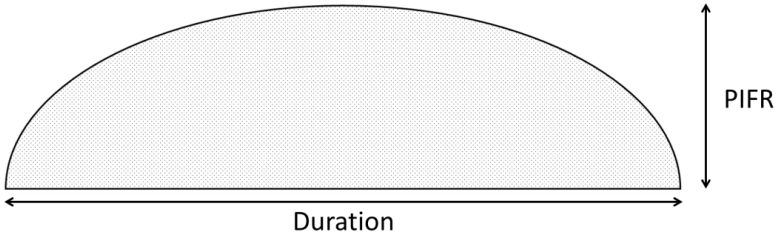

Figure 2.4. Area of semi-ellipse from which the volume or inspiratory capacity (IC) of an inhalation can be calculated.

2.2.2.6. Statistical Analysis

Analysis was carried out using the statistical software Stata SE Version 12. This study was designed as a repeated measures study due to the fact that the samples were not independent. A generalized least squares (GLS) regression model, which accounts for random effects intercept at the subject level, was used to compare the acoustic parameters of MA, MAD, RMS and P_{ave} with measured PIFR and IC. The GLS model takes into account the correlation between the observations when calculating the regression model and was thus deemed appropriate for analysis of the data in this study.

2.2.3. Validation of an acoustic method for estimating inspiratory flow rate and volume from an inhaler using acoustic measurements in a respiratory disease cohort

2.2.3.1. Participants

One hundred and ten subjects from a population of patients with asthma, COPD, lung cancer, neuromuscular disease, other respiratory disorders and non-respiratory disorders were recruited by clustered and stratified sampling. All participants were either on inhaled medications as part of their treatment regimens or received training on how to use a Diskus™ inhaler. Patients were recruited from different clinics in Beaumont Hospital, Dublin, Ireland. There were no specific exclusion criteria for this study apart from capacity to comply with instructions and age (excluded if less than 18 years). Informed consent was obtained for the study with explanations of the study protocol. Demographics and baseline lung function by spirometry were recorded.

2.2.3.2. Ethical approval

The study was approved by the local Hospital Ethics Committee (ERC/ IRB 13/36).

2.2.3.3. Apparatus used

The construction of the airtight container with the associated Diskus™ inhaler, INCA™ ™ device and spirometer connection used in these studies has been described previously in section 2.2.2.3.

2.2.3.4. Study protocol

Patients were instructed to exhale gently to functional residual capacity and then inhale at maximal flow rate and duration. Each patient performed this manoeuvre until two consecutive PIFR readings were within 20% of each other.

2.2.3.5. Data analysis

The audio files recorded from the subjects were subsequently analysed using Audacity version 2.0.5 and MATLAB_R2013b software packages to determine the value of amplitude and duration of each inhalation. Regression analysis (linear, polynomial and rational) was performed to determine the relationship between measured Diskus^{TM} PIFR and each amplitude parameter described in the prior section (MA, MAD, RMS and $P_{ave,\ 300\text{-}600\ Hz}$). The parameter with the greatest degree of correlation was chosen to calculate acoustic PIFR.

2.2.3.6. Statistical analysis

Statistical analysis was done using MATLAB_R2013b and STATA version 13. Creating binary dependent variables using threshold values for measured PIFR, sensitivity and specificity analysis was done comparing acoustically-determined PIFRc with spirometrically-determined PIFRm. Receiver operating characteristic (ROC) curves were constructed and the value of acoustically-determined PIFRc at which the maximum number of inhalations was correctly classified was determined and presented in tabular form.

2.2.4. Correlation of inhalation acoustics from a Diskus™ DPI with in vitro drug delivery

2.2.4.1. Apparatus

In vitro deposition and aerodynamic particle size of the delivered dose from the Diskus™ DPI was characterized using the Next Generation Impactor (US Pharmacopoeia 601, Apparatus 5).(147) The NGI was used with a pre-separator and cups 1-8. A high capacity vacuum pump [HCP4, Copley Scientific, UK] and Critical Flow Controller [TPK 2000, Copley Scientific, UK] were attached to the air intake port. Impaction cups 1-5 were lined with filter papers wetted with 2 ml of a mixture of methanol: acetonitrile: water (25:25:50) and cups 6-8 were coated with 2 ml of solvent only to prevent particle bounce and re-entrainment.(148)

Two Diskus™ inhalers were used in this study: salmeterol 50 µg/ fluticasone 250 µg and salbutamol 200 µg. An INCA™ audio recording device was attached to each inhaler so that acoustic recordings of each inhalation were obtained.

2.2.4.2. Experimental conditions

The study variables were flow rate (PIFR) and duration of inhalation. The critical flow controller was adjusted to achieve flow rates of 30, 60 and 90 l/min at 2, 4 and 6 second durations. Testing was performed in duplicate at each study condition for both inhalers. For each determination, 5 individual doses were aerosolized into the induction port via a mouthpiece adaptor. The active ingredients were quantitatively recovered from the induction port (throat), pre-separator, and cups 1-8.

2.2.4.3. Chromatographic analysis of active ingredients.

High performance liquid chromatography (HPLC) analysis was performed using a Waters Alliance Separations module 2690 [Waters Corporation, MA, USA] equipped with a temperature programmable autosampler and Waters 2996 PDA detector [Waters Corporation, MA, USA]. Chromatographic data was recorded and integrated using Waters Empower chromatography software [Waters Corporation, MA, USA] and quantified using external standards. HPLC conditions for salbutamol sulphate,(149) and fluticasone propionate / salmeterol xinafoate are detailed in Table 2.1.

Analytical method validation was demonstrated for both methods with regard to accuracy, precision, specificity and linearity as per International Conferences on Harmonisation (ICH) guidelines.(150) The limits of detection for salbutamol, fluticasone and salmeterol peaks were 0.045, 0.032 and 0.014 µg / mL, respectively, while the LOQ values for the same three peaks were 0.136, 0.101 and 0.042 µg / mL, respectively.

Table 2.1: Details of high performance liquid chromatographic techniques used for quantification of salbutamol sulphate, fluticasone propionate and salmeterol xinafoate.

Active Ingredient	**Mobile Phase (per 1 L)**	**Flow Rate (mL / min)**	**Column Details**	**Injection Volume**	**Detection Wavelength**
Salbutamol sulphate	600mL - methanol 400mL - deionised water 1g - sodium dodecyl sulphate	1.5	Waters Nova-Pak® C18 5µm 3.9×150 mm,	100 µL	276nm
Fluticasone propionate / salmeterol xinafoate	500mL - 50mM ammonium phosphate pH2.4 1mL - triethylamine 250mL - methanol 250mL - acetonitrile	1.2	Varian Pursuit XRs C18 3µm 4.6 x 150 mm,	200 µL	252nm

2.2.4.4. Measures and data analysis

The total emitted dose (TED) was determined as the sum of the total drug recovered from the Throat, PS, and cups 1-8. This was averaged for each study condition. The fine particle dose (FPD), i.e. cumulative dose less than particle size 5 μm, was calculated by interpolation on a log-probit plot using pre-specified stage cutoffs at each flow rate. Fine particle fraction (FPF) was calculated by expressing the FPD as a percentage of the label claim dose. The upper airway dose (UAD) corresponded to the cumulative dose above an aerodynamic particle size of 5 μm. Flow rate (PIFRc) was calculated from the acoustic parameters using Equation 5. Mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were also calculated at each study condition for both formulations using published methods.(151, 152)

2.2.4.5. Statistical analysis

Statistical analysis was performed using Stata version 13 and MATLAB_R2013b. Multivariate regression analysis was performed using TED, FPF and UAD as dependent variables and PIFR, Duration, PIFRc and acoustic duration as independent variables. Bar graphs of TED, FPF, and UAD for both formulations were generated, grouping by PIFR and duration. The regression effect size (η^2) was calculated for PIFR and duration in each model. Coefficients of variation (CVs) were determined for PIFRc at different levels of measured PIFR and for acoustic duration at different levels of preset inhalation duration to analyze our method precision.

2.2.5. Correlation of inhalation acoustics from a Diskus™ Dry Powder Inhaler with in vivo drug delivery

2.2.5.1. Participants and ethical approval

This study was approved by the local Hospital Ethics Committee (ERC/ IRB 13/53). Ten healthy volunteers were recruited; demographics are shown in Table 4.15 on page 170.

2.2.5.2. Apparatus

An INCA™ acoustic recording device was attached to a 200 µg salbutamol Diskus™ with a hot-wire anemometer [FS5, IST, Switzerland] inserted into an air intake port of the Diskus™. The hot-wire anemometer produced a voltage output which was calibrated against flow rate using a vacuum pump.

2.2.5.3. Sample collection and processing

Blood samples were collected in 7.5 ml serum separator tubes and allowed to coagulate for 20 minutes. Tubes were then centrifuged at 5000 *g* for 15 minutes and 2-3 ml of serum pipetted into vials for storage at -20 ^{o}C.

2.2.5.4. Enzyme linked immunosorbent assay for determination of serum salbutamol concentration

Serum concentration of salbutamol was determined using a competitive Enzyme Linked Immunosorbent Assay [MaxSignal® Salbutamol ELISA Test Kit (Reference 1022-01), New Market Scientific, UK]. Limit of detection for serum/ plasma was 0.25 ng/ml and the assay was linear in the range of 0.05 ng/ml to 10.0 ng/ml. Total assay imprecision was determined to be 14% with recoveries between 85-115%. To account for interference between protein components in the serum and the assay, the baseline sample concentration was subtracted from timed samples.

2.2.5.5. Study protocol

Preliminary pharmacokinetic profiling showed serum peaks at 20 min and at 2-3 hours post-inhalation (Figure 2.5). The sampling time of 20 minutes was used for the comparative study below because this has been reported to represent pulmonary absorption.(37)

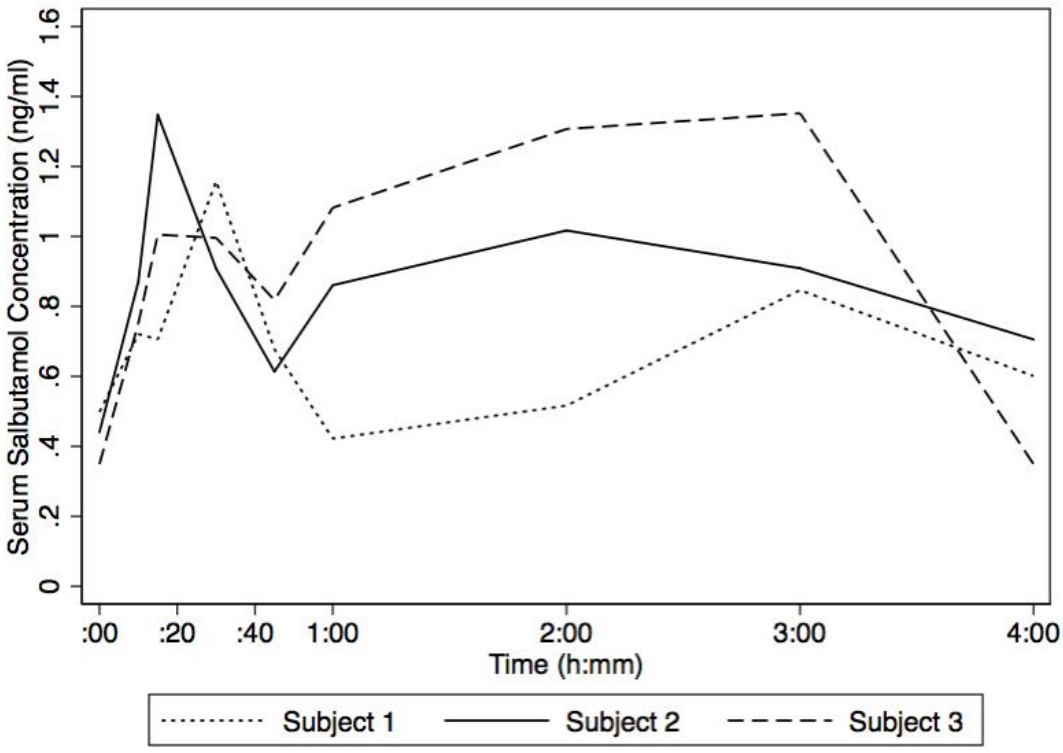

Figure 2.5: Line graph showing serum drug concentration versus time post-inhalation of a 200 microgram dose of Salbutamol via Diskus^{TM} inhaler for three healthy individuals. Note the two distinct peaks in drug concentration at 20-25 minutes and at 2-3 hours.

Due to the wide inter-subject variation in metabolism of salbutamol and other similar compounds, we used each subject as his/ her own control to determine the effect of flow rate and duration of inhalation on peak concentration. Each subject was asked to perform a single inhalation at maximal effort [PIFR ≥ 60

l/min] and duration from the study apparatus. This was followed by a 10 second breath hold and then a mouth rinse to reduce gastro-intestinal absorption of salbutamol. A previous study has shown this to be an effective method.(37) Blood samples were collected at time zero and at 20 minutes. This was followed by at least a 24-hour washout period. The procedure was repeated at a low flow rate [PIFR <60 l/min] and duration (≤ 50% of maximal duration) after this washout period.

2.2.5.6. Statistical analysis

Statistical analysis was done in STATA version 13. PIFR and inhalation duration were determined both from the hot-wire anemometer and from the INCA^{TM} device and correlated for each inhalation. A line graph was done for each subject and an overall regression model was developed using peak concentration as the dependent variable and measured PIFR, duration, calculated PIFR and acoustic duration as independent variables.

2.3. Development and validation of an acoustic method to detect and quantify the effect of exhalation into a Dry Powder Inhaler

2.3.1 Impact of exhalation on delivered dose

2.3.1.1. Dosage uniformity analysis

To validate the in vitro method of removing drug from the Diskus™ DPI, the Dosage Uniformity Sampling Apparatus (DUSA) was used to determine the delivered- dose uniformity from a salmeterol/fluticasone 50 mcg/ 250 mcg Diskus™ DPI (US Pharmacopoeia 601).(147) The Diskus™ DPI was not subject to any exhalations. Ten replications were performed. The target dosage uniformity was 9 of 10 results between 75% and 125% and no more than 1 of 10 results between 65% and 135%.(147)

2.3.1.2. Experimental setup and protocol

To recreate the effect of an exhalation with dry air, a high capacity airflow pump and critical flow controller (air valve) were connected in series to a glass adaptor (mouthpiece) that mimicked the oropharynx (Figure 2.6 – Path A). A salmeterol/fluticasone 50 mcg/ 250 mcg Diskus™ DPI was also used. Relative air humidity was determined using a Testo 410 Humidity Meter [Testo, Hampshire, UK]. For Path A relative air humidity was measured as 28%.
To recreate the effect of an exhalation containing humid air, the high capacity airflow pump and critical flow controller were connected in series with a three-outlet round bottom flask placed in an inductive heater, as shown in Figure 2.6 - Path B. The outlet of the round bottom flask was attached to the glass adaptor. Relative air humidity for Path B was measured as 80%, analogous to the relative humidity of actual exhaled air.(153) Detailed pictures of the equipment used are shown in Figure 2.7.

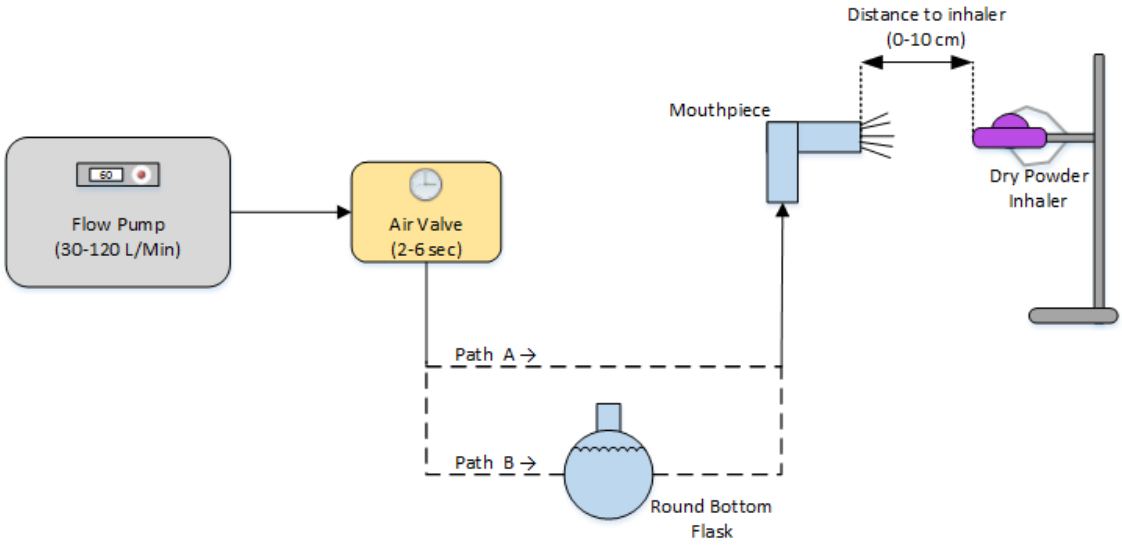

Figure 2.6: Experimental setup used to investigate the impact of exhalations on drug delivery in a dry powder inhaler. Air was propelled at various flow rates and durations through variable flow paths. Path A represents dry air at a relative humidity of 28% and Path B included a round bottom flask filled with boiled water to bring the humidity of the air to 80% relative humidity. Finally the distance between the artificial mouthpiece and the inhaler mouthpiece was also varied.

A **B** **C**

Figure 2.7: Images of glassware, heating device and clampstand used to simulate humidified exhalations into the Diskus^{TM} inhaler mouthpiece.

Dry air (relative humidity of 28%) was blown at the inhaler at flow rates of 30, 60, 90, 120 l/min, for durations of 2, 4, 6 seconds and at distances of 0, 5, 10 cm from the inhaler. Each trial was completed three times in total for all of the conditions (36 variations x 3 runs). After each trial, the inhaler was connected to the DUSA apparatus and the delivered dose was determined. This corresponds with Path A as shown in Figure 2.6.

The round bottom flask was three-quarters filled with distilled water and heated to boiling point to obtain humidified air (relative humidity of 80%). Air travelled on Path B, as demonstrated in Figure 2.6, for this section of testing. The above procedure was repeated at varying flow rates, distances and durations and repeated three times for each condition (36 variations x 3 runs). Finally the delivered dose was determined post-exhalation using a DUSA.

The DUSA apparatus was connected to a high capacity vacuum pump [HCP4, Copley Scientific, Nottingham, UK] and Critical Flow Controller [TPK 2000, Copley Scientific, Nottingham, UK]. The Flow Controller was operated at 60 l/min at a pressure drop of 4 kPa for a duration of 4 seconds

2.3.1.3. Data Analyses

Data Analysis was carried out in Stata version 13. Multivariate regression analysis was performed to investigate what exhalation factors had a significant effect on drug delivery. Eta squared and partial eta squared values were calculated to interpret the individual effect size for the four exhalation factors. Eta squared measures the proportion of the total variance in a dependent variable that is accounted for by variation in the independent variable. It is the ratio of the between groups sum of squares to the total sum of squares. Partial eta squared measures the proportion of variance accounted for by an effect to the proportion of variance accounted for by the same effect plus its associated error variance (i.e., the effects of other independent variables and interactions are partialled out).

2.3.1.4. Particle size distribution of emitted dose

Testing was carried out to investigate the effect of humid air exhalations on the particle size distribution of the total emitted dose (TED) for the Diskus™ DPI. To investigate this, the TED and fine particle fraction (FPF) from a Diskus™ that had previously been subjected to an exhalation were compared to TED and FPF obtained from a Diskus™ that was not subject to an exhalation.

In vitro drug deposition and aerodynamic particle size of the delivered dose from the Diskus™ DPI was characterized using a Next Generation Impactor (NGI) (US Pharmacopoeia 601, Apparatus 5).(147) The NGI was used with a pre-separator and cups 1-8. A high capacity vacuum and critical flow controller were attached to the air intake port. Inhalations were performed at a flow rate of 60 L/Min, pressure drop of 4 kPa and duration of 4 seconds. NGI impaction cups 1-5 were lined with filter papers and with 2 ml of a mixture of methanol: acetonitrile: water (25:25:50). Cups 6-8 were coated only with 2 ml of solvent. This was to prevent particle bounce and re-entrainment.(148)

To investigate the effect of exhalations on drug delivery in a DPI, the test setup explained previously was employed to exhale air at a flow rate of 60 l/min, for a duration of 4 seconds and using air with a relative humidity of 80% at a Diskus™ DPI. Exhalations were carried out on five separate Diskus™ DPI's in order to preserve the possible humidity effect from each trial. A regular Diskus™ DPI that was not subjected to any exhalations was used to compare the effects of the exhalations.

The TED was determined as the sum of the total drug recovered from the throat, pre-separator, and cups 1-8 of the NGI. This was averaged for each study condition. The Fine Particle Dose (FPD), i.e. cumulative drug dose less than particle size 5 μm, was calculated by interpolation on a log-probit plot using pre-specified stage cutpoints at each flow rate. The Upper Airway Dose (UAD) corresponded to the cumulative drug dose above an aerodynamic particle size of 5 μm. Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) were also calculated at each study

condition for both formulations using published methods.(151, 152) The TED, FPD and UAD for the standard emitted dose and the post-exhalation emitted dose were compared.

2.3.1.5. Measurement of salmeterol and fluticasone

The method described in section 2.2.4.3. was used to measure salmeterol and fluticasone concentrations using HPLC-UV.

2.3.2. Relationship between simulated exhalations and acoustic features

The INCA™ acoustic recording device was attached to the Diskus™ inhaler during experimentation to investigate the effect of different exhalation factors on delivered dose, and if acoustic features could be used as a means to analyse exhalations. Temporal and spectral features of the exhalation signal were analysed to investigate the feasibility of using acoustics to analyse exhalations during inhaler use. The mean absolute deviation (MAD) and average power (P_{ave}) of the exhalation signal were computed and compared to the flow rate of the exhalations and to the distance of the exhalations from the inhaler mouthpiece. Exhalations were divided into 1024 data samples with 50% overlap between successive frames. A Hanning window was used to analyse each frame, while a Fast Fourier Transform (FFT) was used to calculate the power spectral density (PSD). P_{ave} was calculated for frequencies between 300 – 600 Hz. Previous studies have reported that this frequency band shows the best correlation between airflow rate and sound power.(146, 154) MAD is the mean of the absolute deviations from the central value. It was calculated using the following equation:

$$MAD = \frac{1}{n}\sum_{i=1}^{n}|x_i - \bar{x}| \qquad (2.4)$$

Correlations between the simulated expiratory flow rates and distances from the inhaler are presented in the results section.

2.3.2.1. Acoustic method of automatically detecting exhalations

As previously mentioned, exhalations occurring prior to the inhalation step during inhaler use are crucial to detect as they affect pulmonary drug delivery. Previous studies have investigated the detection of exhalations during normal relaxed breathing, in speech and song signals, however, the detection of exhalations recorded during inhaler use have never been investigated in detail.(155, 156) In this study a training database of inhaler audio files was employed to develop an algorithm to automatically detect exhalation events

from non-exhalation events in the audio signals. The algorithm developed was then subsequently tested for sensitivity, specificity and accuracy using a validation dataset of audio files obtained from separate patients.

2.3.2.2. Exhalation Detection Algorithm

Filter-bank energies (FBEs) obtained from calculation of the Mel Frequency Cepstral Coefficients (MFCCs) are used as features to detect exhalations in the audio signals in this study. FBEs are physically meaningful quantities that are known to correlate with human auditory processing.(157) Audio events (exhalation and non-exhalation events) were automatically detected using an adaptive energy threshold in this study (Figure 2.8). Exhalations were segments with higher energy in certain frequency regions compared to other background noises in the audio signals. The FBEs are computed using the following steps:

Signal is first epoched into frames of length 25ms (N_w), which overlap every 10ms.

Calculation of energy spectrum:

$$y(k) = \sum_{n=0}^{N_w-1} x(n)W(n)e^{-j\frac{(2\pi nk)}{N_w}}; \quad 0 \le k \le N_w \tag{2.5}$$

where x(n) is the input inhaler signal and W(n) is a Hamming window. The energy spectrum is subsequently given by:

$$X_k = |y(k)|^2; \qquad 0 \le k \le K \tag{2.6}$$

where K is taken equal to Nw/2, as only half the spectrum is considered. Using a lower frequency limit of 0 Hz and an upper frequency limit of 4000 Hz, 20 filter banks were created using the following equation described in (158):

$$M(f) = 2595log_{10}\left(1 + \frac{f}{700}\right) \tag{2.7}$$

The energy in each filter bank is then calculated:

$$E_j = \sum_{k=0}^{K-1} \emptyset_j(k) X_k ; \;\; 0 \leq j < J \tag{2.8}$$

where J, which equals 20, is the number of triangular filters ($\emptyset_j$) used.

FBE channels are then normalized between 0 and 1. To remove short duration noise artefacts in the signal, the root mean square (RMS) amplitude of 50 ms frames, which overlap every 10ms, are calculated.

To detect exhalation events the average energy in filter banks 8-10 is calculated. It was found from empirical observation in the training dataset that the energy in these three filter banks was higher for exhalations in comparison to other audio sounds obtained during inhaler use. These filter banks correspond with triangular filters starting at 620 Hz and ending at 1197 Hz. Comparing the average energy in these three filter banks to the average FBE in 20 channels provides a difference waveform (DW) that can be used to automatically detect exhalations.

$$DW = \overline{FBE}_{8-10} - \overline{FBE}_{1-20} \tag{2.9}$$

An adaptive energy threshold was used to automatically detect and segment exhalations events in the DW signal. The mean of all positive values of the DW signal was computed and an adaptive threshold was set that was 20% higher than this mean value. Potential exhalations less than 200 ms in duration were discarded in order to avoid the false detection of sudden noise artefacts. This method allowed the successful classification of exhalations in the training dataset.

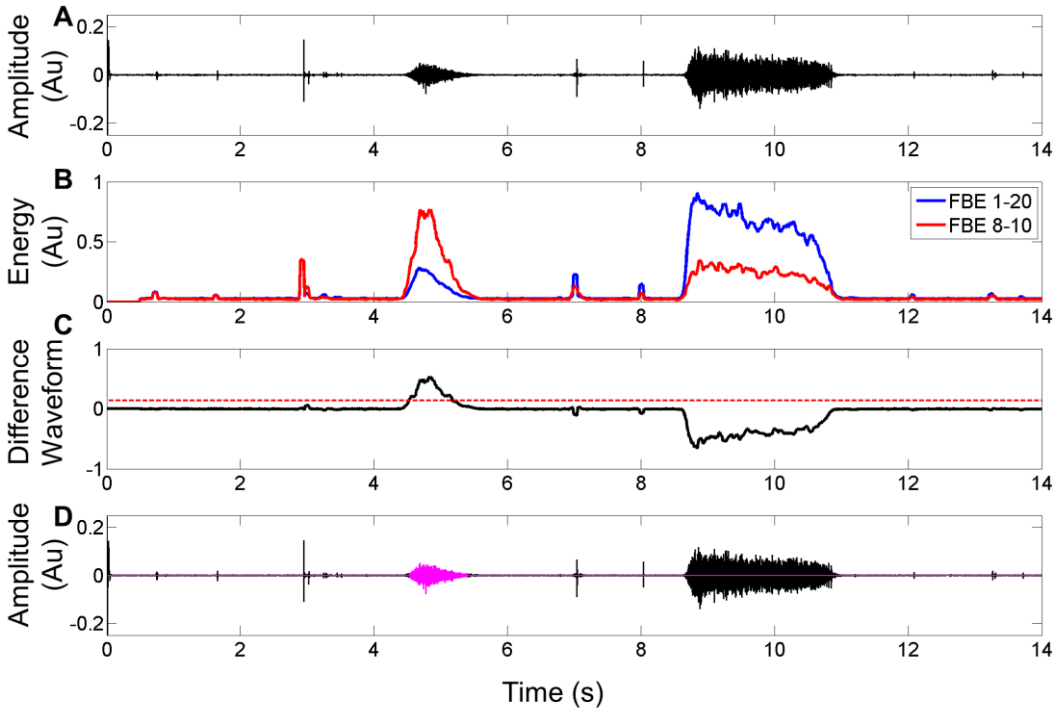

Figure 2.8: (a) Inhaler audio signal containing exhalation at 5s and inhalation at 9-11s, (b) average FBE for channels 1-20 (red) and channels 8-10 (red), (c) difference waveform (FBE_{8-10} – FBE_{1-20}) and adaptive threshold (dashed red line) and (c) inhaler audio signal with automatically detected exhalation colored in magenta.

2.3.2.3. Data and Statistical Analyses

Acoustic signal processing and statistical analyses were performed using MATLAB_R2013b. The training database consisted of 50 audio files obtained from 10 patients with asthma and COPD using a Diskus™ DPI in uncontrolled real world scenarios. Audio signals were obtained from the INCA™ acoustic recording device. The training database was employed to decide which specific FBE channels contained the largest amount of energy for exhalations and in the design of the adaptive energy threshold. The validation dataset comprised of a random cross-section of inhaler audio files obtained from 22 separate asthma and COPD patients. Similar to the training database, the audio files were obtained in uncontrolled real world environments using the aforementioned INCA™ acoustic recording device. Five audio files were

randomly selected from each patient to give a total of 110 audio files in the validation dataset.

Two experienced respiratory clinicians independently classified each audio file in the validation dataset using visual and aural methods. The classification of the audio files by the respiratory clinicians was used as the gold standard method of exhalation detection. Exhalation detection performances of the algorithm were compared to that of the gold standard method and calculated using sensitivity, specificity and accuracy values.

Cohen's kappa statistic (*k*) was used to measure the agreement between the two respiratory clinicians. It was also employed to measure the level of agreement between the gold standard classification of exhalations and the proposed automatic detection method. The guidelines for interpreting *k* are as follows:

Table 2.2: Interpretation of Cohen's Kappa statistic (k)

Cohen's Kappa Statistic *(k)*	**Interpretation**
<0	Less than chance agreement
0.01-0.20	Slight agreement
0.21-0.40	Fair agreement
0.41-0.60	Moderate agreement
0.61-0.80	Substantial agreement
0.81-1	Almost perfect agreement

2.3.3. Acoustic method of assessing exhalations during inhaler use

A Diskus™ inhaler with an INCA™ acoustic device attached was clamped to a stand. Healthy subjects performed subjectively variable exhalations at distances of 0 cm, 5 cm and 10 cm from the mouthpiece of the inhaler in locations located above, below and directly into the mouthpiece of the Diskus™ inhaler. Exhalations were also performed with a mouth seal at subjectively variable high and low flow rates. Forty exhalations from three volunteers were analysed (training dataset) to develop an algorithm for determining the distance of the exhalation from the inhaler mouthpiece and the expiratory flow rate of the exhalation. Exhalations were divided into 1024 data samples with 50% overlap between successive frames. A Hanning window was used to analyse each frame, while a Fast Fourier Transform (FFT) was used to calculate PSD. P_{ave} in the frequency bands 20-40 Hz (P1), 40-70 Hz (P2) and 70-150 Hz (P3) was calculated. The MAD of the amplitude of the exhalation signals was also calculated using equation 2.1.

The following three equations were derived from the training dataset to classify different aspects of exhalations:

$$Mouthseal\ Test = MAD > 0.002\ \&\frac{P2}{P3} > 0.91\ \&\frac{P1}{P3} > 0.91 \quad (2.10)$$

$$Significant\ Exhalation = MAD > 0.003\ \ or\ Mouthseal\ test = 1 \quad (2.11)$$

$$Exhalation\ at\ 5cm = MAD > 0.002\ \&\frac{P1}{P2} < 0.975 \quad (2.12)$$

Significant exhalations were classified as any exhalation performed at a distance of 0 cm or 5 cm from the inhaler mouthpiece, directly at the inhaler mouthpiece or any exhalation performed with a mouthseal. Any exhalation directly at the acoustic recording device was also classified as being significant. We also tested the sensitivity and specificity of our method for distinguishing between an exhalation performed at 0 cm and one performed at 5 cm.

To test the robustness of equations 2.10 - 2.12 in classifying exhalations, we obtained a validation dataset of fifty exhalations from 4 healthy subjects. Temporal and spectral features of the exhalation signal were extracted and employed to classify the exhalations. Classification results were compared with documented conditions for the exhalations in the validation dataset to obtain sensitivity and specificity values of the method in determining significantly detrimental exhalations.

2.4. Does orientation of the Diskus™ inhaler affect available dose for delivery?

A 200mcg salbutamol Diskus™ inhaler was clamped in a horizontal position (designated 0°) and a petri dish was placed directly beneath the mouthpiece of the inhaler (Figure 2.9).

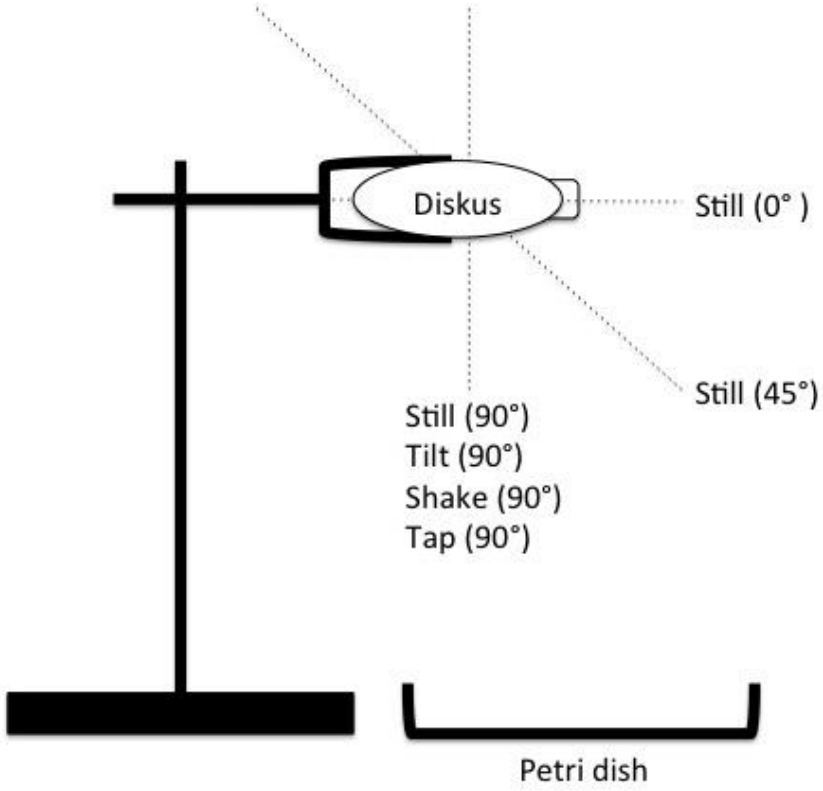

Figure 2.9: Experimental setup to investigate the effect of Diskus™ orientation on drug removed from the inhaler. The Diskus™ was position at 0°, 45° and 90° and the drug removed was collected in the petri dish, dissolved and analysed.

A dose was actuated and the drug released from the inhaler was allowed to collect in the petri dish. The contents of the petri dish were dissolved in 5 ml of methanol and the sample was analysed by HPLC-UV using the method described in 2.2.4.3. above.

The Diskus™ inhaler was then held at 45° and 90° and the experiment above repeated. The Diskus™ was then clamped at 90°, a dose actuated and the inhaler was either tapped or shaken. Five replicates were performed for each experimental condition.

The mean drug lost from the inhaler under each experimental condition was calculated and compared with the drug lost in the horizontal position using a t-test.

2.5. The impact of breath holding duration on drug delivery

2.5.1. Study protocol

This was a prospective study of seven healthy volunteers using a salbutamol 200mcg Diskus™ inhaler. The inclusion criteria allowed for recruitment of healthy participants older than 18 years of age and non-frequent users of salbutamol.

Each participant received Diskus™ inhaler technique training and was assigned to do a control 'phase', consisting of six doses of the drug taken six hours apart with correct technique and a breath hold duration of 10 seconds. Blood samples were collected before and 25 minutes after doses one and six. Blood samples were collected in 7.5 ml serum separator tubes and allowed to coagulate for 25 minutes. Tubes were then centrifuged at 5000 *g* for 15 minutes and 2-3 ml of serum pipetted into vials for storage at -20 ^{o}C.

This was followed by a washout period of at least 3 days (12 half-lives). The volunteers then repeated the above procedure, this time with low breath hold duration of approximately 4 seconds.

The INCA™ device was used to monitor time of inhaler use, inhaler technique and breath hold duration. The audio signature of each breath was analyzed with the software Audacity. At the end of each phase, the inhalers were collected and stored at the lab.

2.5.2. Measurement of serum salbutamol

Serum salbutamol concentration was measured using a method adapted from Sidler-Moix et al.(159) Salbutamol sulphate was purchased from Sigma Aldrich [St. Louis, MO, USA] and salbutamol-d4, the internal standard (IS), was purchased from Toronto Research Chemicals [Toronto, Ontario, Canada]. All chemicals and solvents were of analytical grade and used as received.

Protein precipitation was used to prepare samples for subsequent analysis. Briefly, a 300 µl aliquot of serum was added to Eppendorf tubes, followed by 25 µl of internal standard (500 ng/ml of salbutamol-d4 in acetonitrile) and the tubes were vortexed for 30 seconds. 0.9 ml of acetonitrile was added to each tube and the mixture vortexed for 2 minutes and then centrifuged at 5000g for 10 minutes. The supernatant was transferred to new tubes and was evaporated to dryness under nitrogen gas at room temperature. The residue was reconstituted in 150 µl of 0.5% formic acid in water and vortexed for 2 minutes. The solution was then subjected to further analysis as outlined below.

The high-performance liquid chromatography system was a Waters Alliance 2795 separation module with quaternary pump and autosampler, controlled by Waters MassLynx software [Waters Corporation, MA, USA]. The separations were carried out on a 2.1x50 mm Atlantis T3 3 µm analytical column [Waters Corporation, MA, USA]. The chromatographic system was coupled to a Waters Quattro Micro triple quadrupole mass analyzer with an Electrospray Ionization (ESI) source.

The mobile phase used consisted of 10 mM ammonium formate in ultrapure water containing 0.1% FA (= solution A) and acetonitrile with 1% FA (solution B). The following stepwise gradient elution protocol was used:

Table 2.3: Gradient elution protocol for measurement of serum salbutamol by HPLC-ESI-MS/MS

Time (min)	Flow Rate (ml/min)	Solvent A	Solvent B
0.0	0.3	95	5
3.0	0.4	95	5
7.0	0.4	20	80
8.0	0.4	20	80
9.0	0.3	95	5
10.0	0.3	95	5

Solvent A = 10mM ammonium formate in ultrapure water containing 0.1% FA; Solvent B = acetonitrile with 1% FA

ESI was set in positive ionization mode and operated at a capillary voltage of 3.5 kV. The source temperature was set at 120°C, the desolvation temperature was set at 350°C and the desolvation gas flow was 650 L/h. The cone voltage was 30 V, the extractor voltage was 2 V and the RF lens voltage was 0.1 V. MS1 and MS2 low and high mass resolutions were set at 15. Ion Energy 1 was 1.2 and Ion Energy 2 was 1.0. Entrance potential was -2 V and exit potential was 2 V. The multiplier potential was 650 V. Mass spectra were acquired in the Multiple Reaction Monitoring mode. The optimal potential settings and the MS/MS transitions were determined by direct infusion into the MS/MS detector of salbutamol and IS solutions separately at a concentration of 10 µg/mL in methanol (Table 2.4).

Table 2.4: Instrument method for determination of salbutamol and salbutamol-d4 by ESI-MS/MS.

Analyte	Precursor (m/z)	Product (m/z)	Collision Energy (eV)	Retention Time (min)
Salbutamol	240.1	147.8	25	4.54
Salbutamol-d4	244.1	151.9	23	4.52

A representative chromatogram is shown in Figure 2.10.

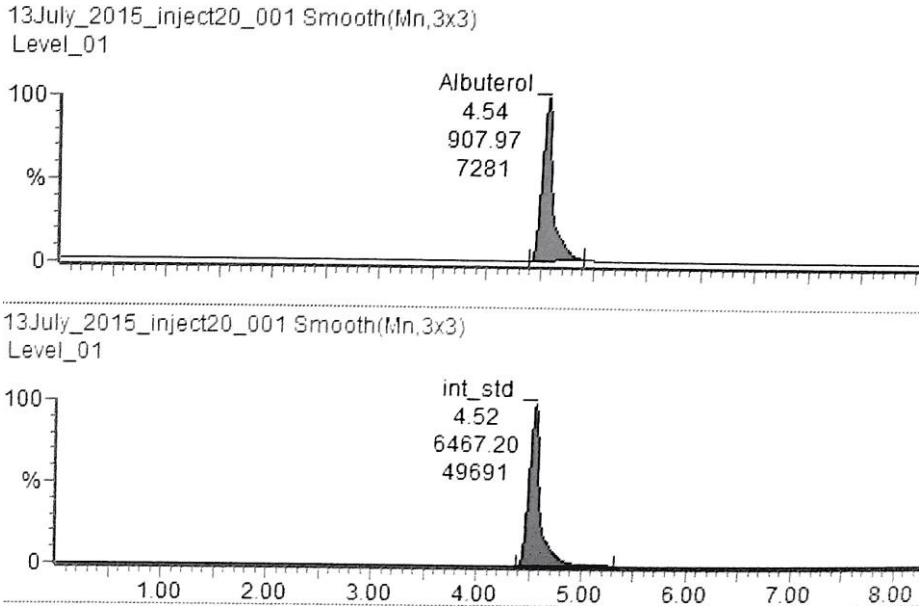

Figure 2.10: Sample chromatogram showing salbutamol (labeled Albuterol) and salbutamol-d4 (labeled int_std) peaks at a retention time of 4.54 and 4.52 min, respectively.

Calibrators were made by spiking blank serum obtained from the blood donation center with known amounts of salbutamol standard at the following concentrations: 10.00, 5.00, 2.50, 1.25, 0.64, 0.32, 0.16, 0.08, 0.04 ng/ml. Quality control samples were also made at the following levels: 7.50, 1.00 and 0.10 ng/ml. A standard curve was fitted using the internal standard response ration method and linear regression with 1/x weighting.

Method imprecision was determined by performing 5 replicates per day for 5 days at all 3 levels of QC. Total imprecision was 12% at the LQC, 6% at the MQC and 5% at the HQC. Recovery studies were performed by spiking known concentrations of salbutamol into the 3 QCs and values were determined to be between 85 and 115% at all QC levels. The limit of detection for the assay was 0.01 ng/ml and the limit of quantification was 0.04 ng/ml.

2.5.3. Data analysis

Statistical analysis was done using Stata version 13. Peak and trough salbutamol levels were compared between the 10 second breath hold and the 4 second breath hold using a box-plot and t-test.

2.6. The impact of missed doses on steady state trough and peak levels

2.6.1. Study protocol

This was a prospective study of seven healthy volunteers using a salbutamol 200 mcg Diskus™ inhaler. The inclusion criteria allowed for recruitment of healthy participants older than 18 years of age and non-frequent users of salbutamol.

Each participant received Diskus™ inhaler technique training and was assigned to do a control 'phase', consisting of six doses of the drug taken six hours apart with correct technique. Blood samples were collected before and 25 minutes after doses one and six. Blood samples were collected in 7.5 ml serum separator tubes and allowed to coagulate for 25 minutes. Tubes were then centrifuged at 5000 *g* for 15 minutes and 2-3 ml of serum pipetted into vials for storage at -20 ^{o}C.

This was followed by a washout period of at least 3 days (12 half-lives). The volunteers then repeated the above procedure, this time missing doses three and four, that is, taking doses 1, 2, 5 and 6. Sampling was again done before and 25 minutes after doses one and six.

The INCA™ device was used to monitor time of inhaler use and inhaler technique. The audio signature of each breath was analyzed with the software Audacity. At the end of each phase, the inhalers were collected and stored at the lab.

2.6.2. Measurement of serum salbutamol

Serum salbutamol concentration was measured using the ESI-LC-MS/MS method outlined in section 2.5.2.

2.6.3. Data analysis

Statistical analysis was done using Stata version 13. Peak and trough salbutamol levels were compared between the control phase and missed dose phase using a connected dot plot and t-test. Results were also compared to expected results based on the half-life of salbutamol and a first-order kinetic model represented by:

$$Concentration, C = Initial\ concentration, C_0 * e^{-0.673* \quad \frac{t}{T}}) \tag{2.13}$$

$$where\ t = measurement\ time\ and\ T = half\text{-}life$$

2.7. Development and validation of an algorithm for combining time and technique of inhaler use into a single metric

2.7.1. Algorithm development

A pharmacokinetic model was developed using features obtained from the INCA^{TM} device:

1) Date and time of use
2) Technique errors
    a. Failure to actuate dose
    b. Exhalation towards the mouthpiece
    c. Low peak inspiratory flow rate
    d. Short breath hold duration
    e. Multiple inhalations (causing short breath hold duration)

The above technique errors were considered significant from the in vitro and in vivo experiments outlined in previous sections.

MATLAB_R2013b and Stata version 13 were used to develop the pharmacokinetic model. The model was based on first-order kinetics for salmeterol, using an estimated drug half-life of 12 hours.

2.7.2. Comparison of algorithm adherence rates with dose counter rate in a patient cohort

Audio recordings from 20 patients were drawn from our INCA^{TM} asthma database in order to compare adherence rates over a period of 1 month between the dose counter on the Diskus^{TM} inhaler and those derived from analysis of the time-stamped acoustic files. Two independent raters reviewed all audio files for the purpose of classification of technique errors. Agreement between raters was 90% (Cohen's kappa). Patients were classified into one of three categories based on the combination of temporal and technique adherence:

Class 0 – Poor overall adherence

Class 1 – Moderate overall adherence

Class 2 – Good overall adherence

Data analysis was done in Stata version 13. Adherence rates were compared using a boxplot and a scatterplot matrix to reveal trends, and Spearman's and Pearson's correlation coefficients with associated p values were determined for all pairwise comparisons. Representative scatterplots and bar graphs showing the pattern of inhaler use over the course of the month are also presented for each subject.

CHAPTER 3: How common are Diskus™ inhaler temporal and technique errors in a community care setting?

3.1. Results

3.1.1. Patient demographics

One hundred and twenty three patients were enrolled for an average of 29.6 days (range: 21-41). After excluding devices that were not returned and device failures, acoustic recordings were available from 103 patients, with 5045 audio files. There were 5228 doses taken according to the dose counter ($p<0.001$ for difference between dose counter and acoustic recordings).

Table 3.1: Patient demographic data from respiratory cohort given INCA™ enabled Diskus™ for one month.

Parameter	Mean or %
Average Age (range)	57.2 (22-91)
Gender (M:F)	46:57 (45:55%)
Diagnosis	65% Asthma
	32% COPD
	3% Other
Smoking History	40% Non-Smoker
	40% Current Smoker
	20% Ex-Smoker
Education Level	42% Primary
	42% Secondary
	15% College

3.1.2. Errors in inhaler technique

3823 (76%) acoustic profiles demonstrated good inhaler technique; a visual profile of the acoustic signal associated with correct inhaler technique is shown in Figure 3.1.

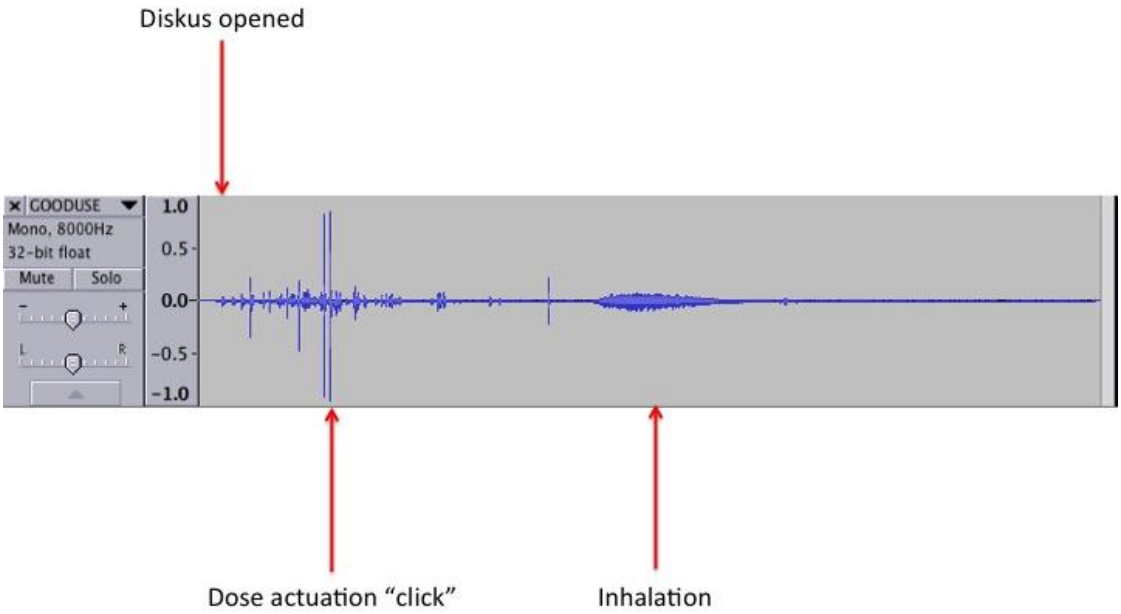

Figure 3.1: Visual representation of acoustic profile showing correct inhaler technique.

Poor peak inspiratory flow rate (PIFR) was the most common inhaler technique error identified; 325 (27%) inhalations were performed with a PIFR< 35 l/min (shown in Figure 3.2).

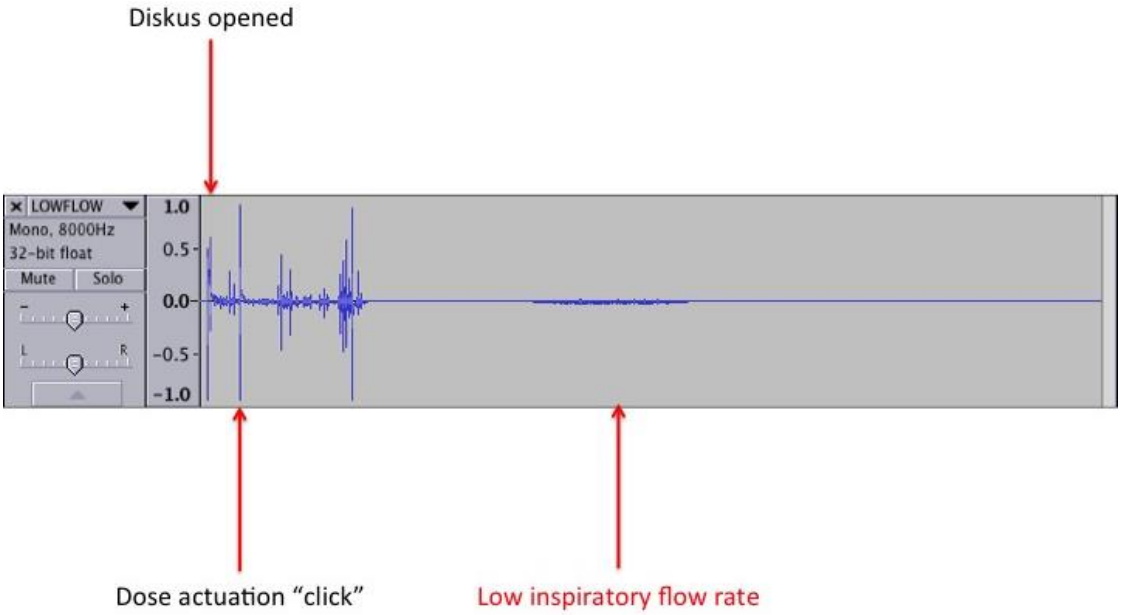

Figure 3.2: Visual representation of acoustic profile showing low inspiratory flow.

Other errors included: dose actuation or blistering with no subsequent inhalation - 229 (19%) audio files; exhalation into the inhaler after dose actuation and prior to inhalation (Figure 3.3) - 217 (18%) events; multiple inhalations (Figure 3.4) - 301 (25%) inhalations; multiple blisters (Figure 3.5) - 72 (6%) of audio files; and rarer events, including cough after inhalation (Figure 3.6), failure to actuate dose (Figure 3.7) and shaking of the inhaler after dose actuation (Figure 3.8).

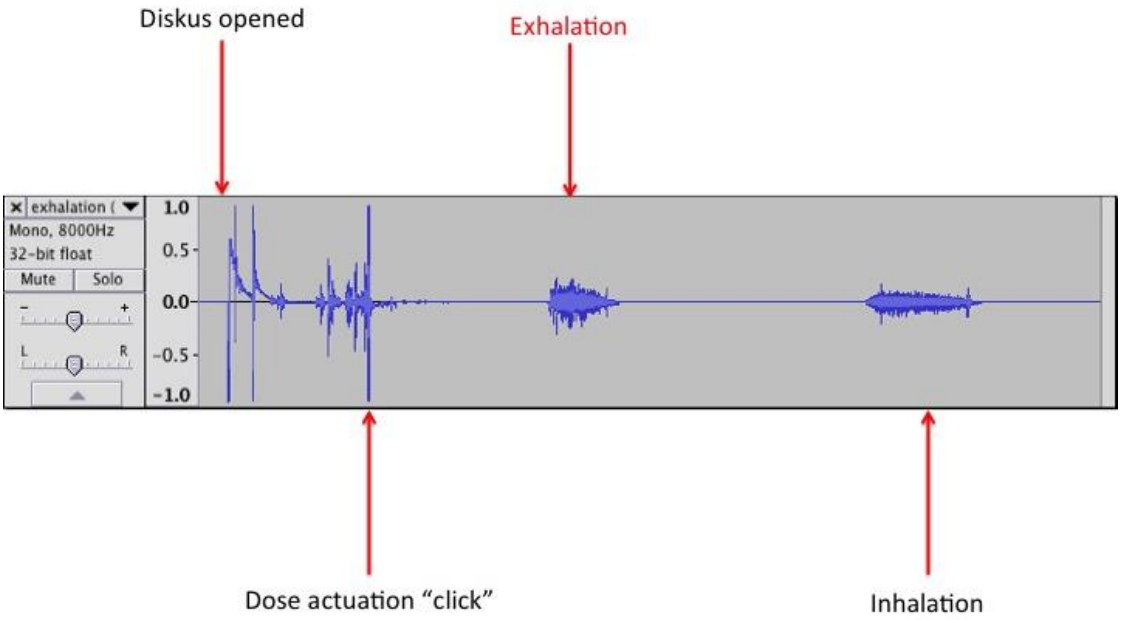

Figure 3.3: Visual representation of acoustic profile showing exhalation directed towards device prior to inhalation.

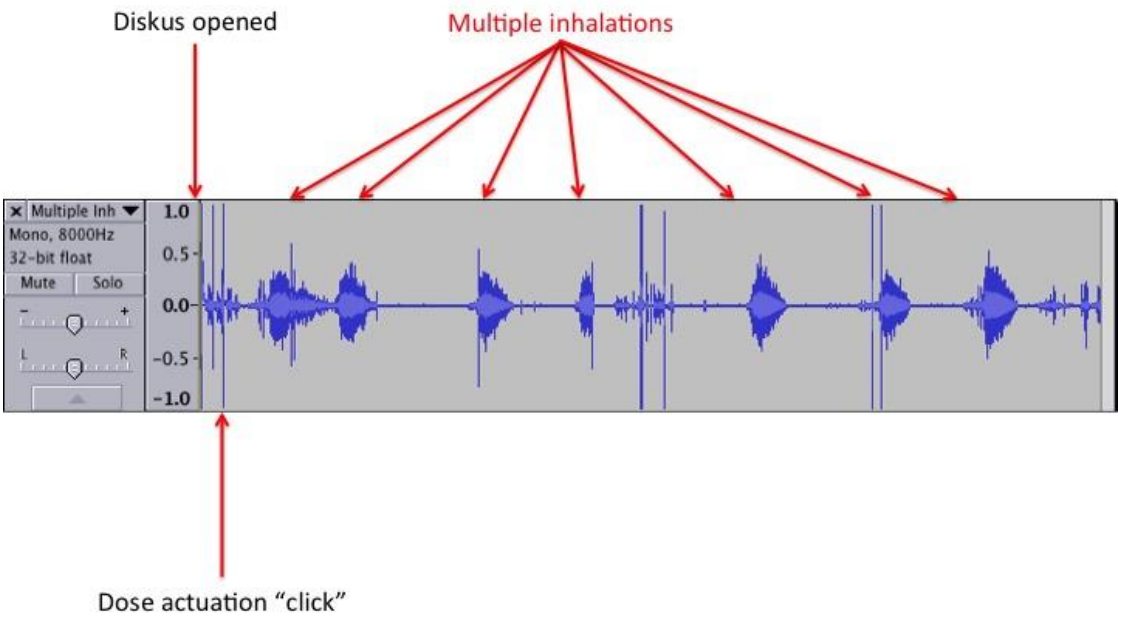

Figure 3.4: Visual representation of acoustic profile showing multiple inhalations.

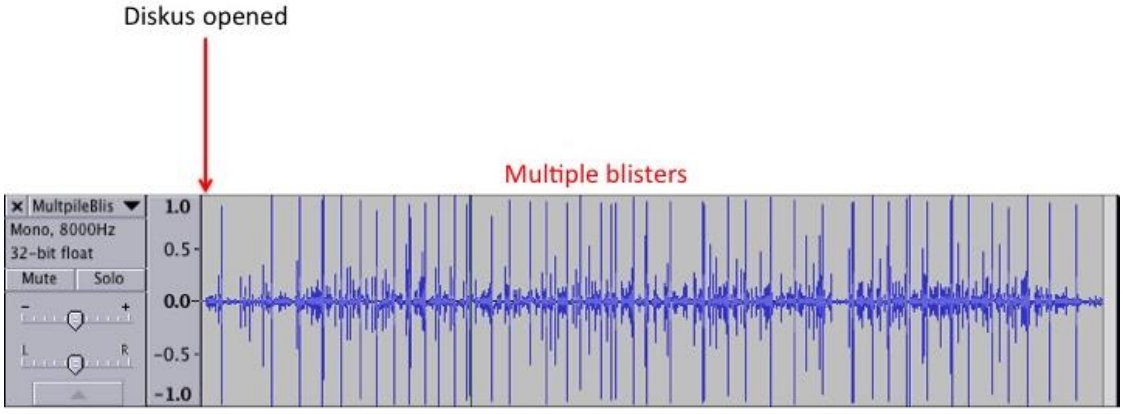

Figure 3.5: Visual representation of acoustic profile showing multiple dose actuations or blisters.

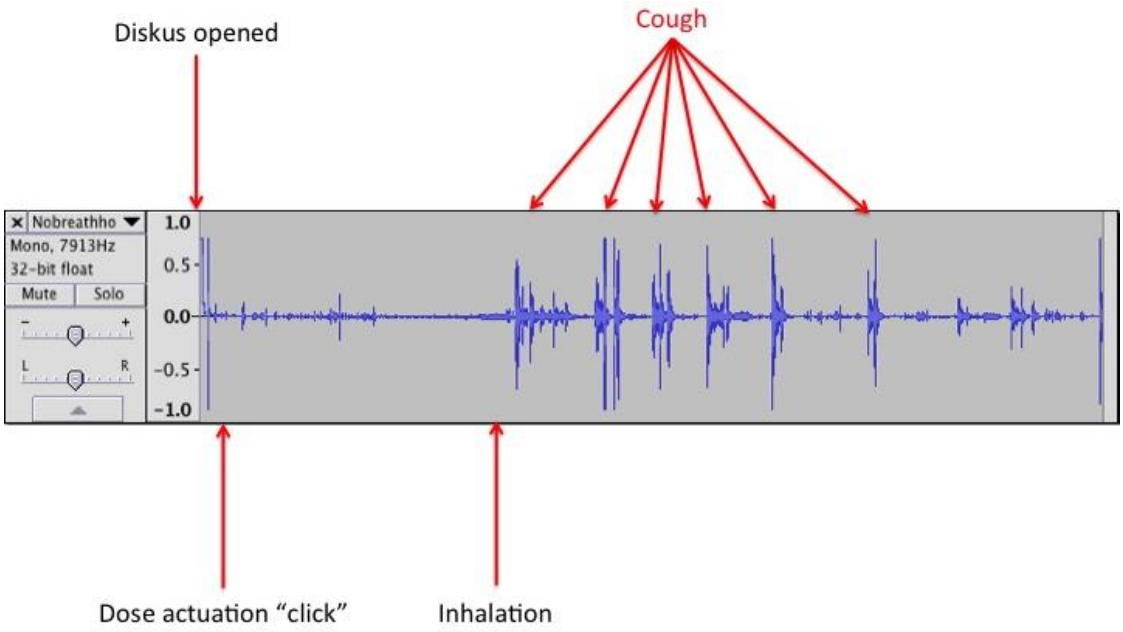

Figure 3.6: Visual representation of acoustic profile showing bout of coughing after inhalation.

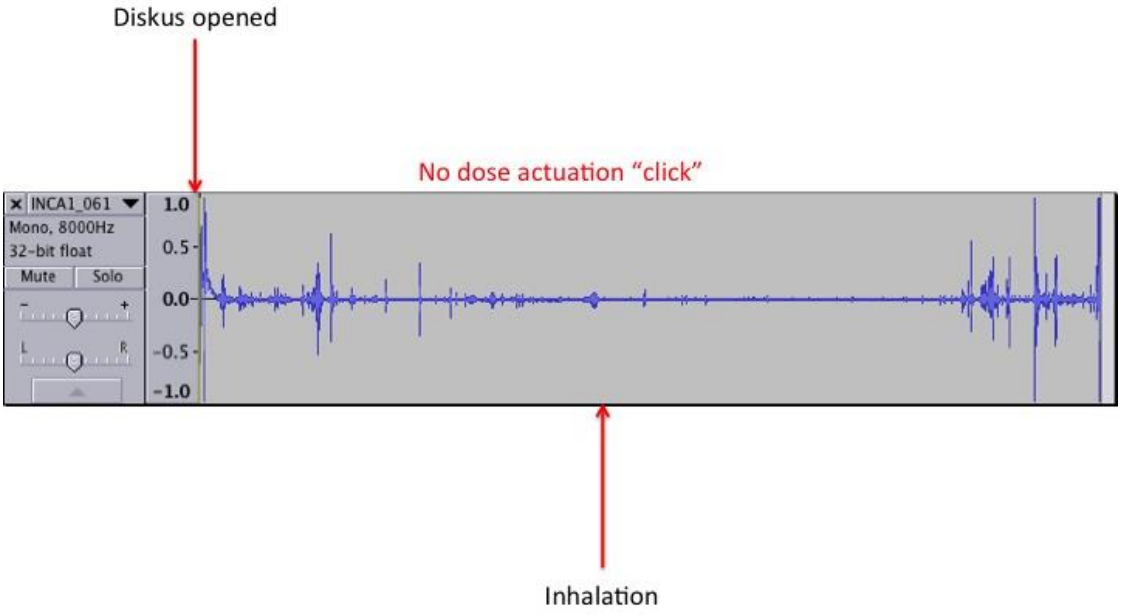

Figure 3.7: Visual representation of acoustic profile showing failure to blister or actuate dose.

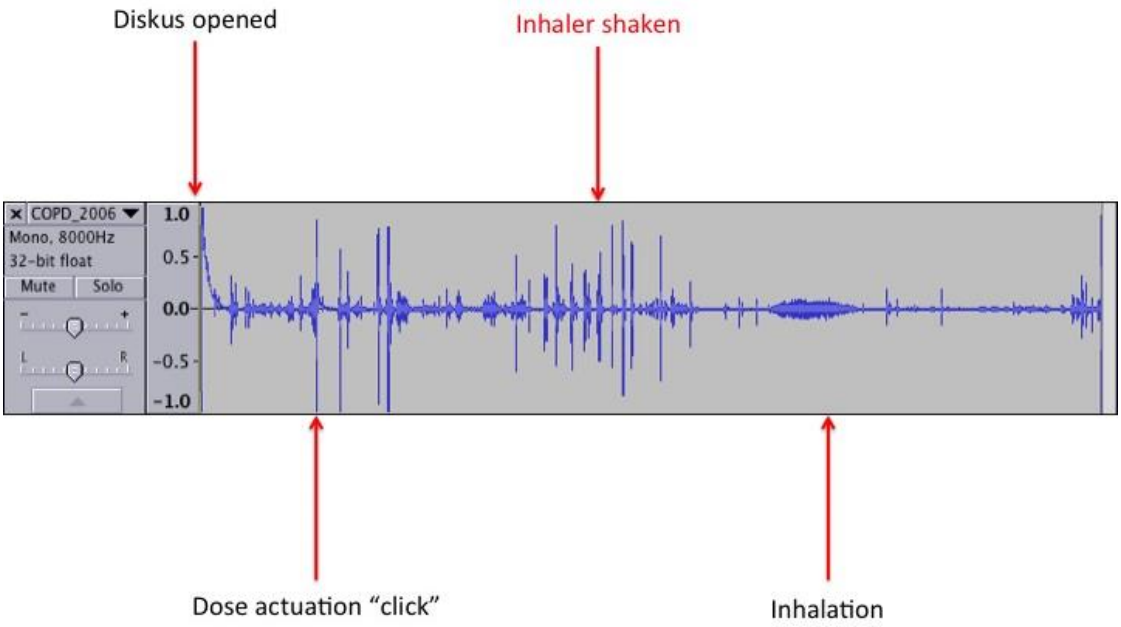

Figure 3.8: Visual representation of acoustic profile showing shaking of inhaler after blister or dose actuation.

While errors were common, ranging from 100% (i.e. a patient who made persistent technique errors) to 0% it is noteworthy that among the studied cohort there was a wide variation in the frequency of inhaler technique user errors and the mean number of errors per person was 12 per 60-dose inhaler (20%). A breakdown of inhaler technique errors observed is shown in Table 3.2.

Table 3.2: Frequency of errors in inhaler handling among the 103 patients.

Instruction	Audio Error	Frequency (% of all errors)	Average per patient (range)
Total number		1204 (24%)	12 (0-60)
Blister			
	No Blister, Inhale Detected	24 (2%)	0.23 (0-4)
	Multiple Blisters	72 (6%)	0.68 (0-23)
	Dose Dumping	36 (3%)	0.34 (0-23)
Breath out deeply away from the inhaler			
	Exhales into inhaler with sufficient energy to displace >30% of dose	**217 (18%)**	**2.17 (0-46)**
Inhale deeply			
	Blister present, No Inhale	**229 (19%)**	**2.28 (0-47)**
	Low PIFR (<35 l/min)	**325 (27%)**	**3.25 (0-60)**
Hold breath for > 5 sec			
	Multiple Inhalations	**301 (25%)**	**3.05 (0-50)**

3.1.3. Errors in time of use of the inhaler

Only twenty-one (20%) patients demonstrated correct technique and temporal adherence. Twenty (19%) individuals used more than 2 doses in a day for at least two consecutive days, with 66 episodes of clustered overdosing, see Table 3.3.

On the other hand, twenty-seven (26%) individuals had at least one incidence of a four half-life interval between doses, leading to 56 sub-therapeutic or non-therapeutic drug intervals. Thirty-eight (37%) individuals missed taking 4 doses over two days, leading to 97 episodes with clustered missed doses.

Table 3.3: Breakdown of different measures of adherence, showing the number of doses expected to be taken over the time, the number of doses actually taken during the study period judged from the dose counter, the number of doses attempted based on the number of audio files, number of doses successfully taken without technique errors, frequency of missed doses and overdoses and the number of technique errors.

	Total	**Average per Patient**	**Range**	**95% CI**
Number of doses expected	6180	60	-	-
Number of doses taken (dose counter)	5228 (85%)	55	12-60	53-57
Number of doses taken (INCA)	5045 (82%)	49	3-67	46-52
Number of doses without technique errors (INCA)	3823 (76%)	34	0-60	30-38
Number of episodes of significant clusters of missed doses	97		0-8	0.63-1.3
Number of episodes of significant clusters of overdoses	66		0-14	0.24-1.0
Number of errors	1204 (24%)	12	0-60	9-15

3.1.4. Variations in inhaler use over time

The rate of adherence over the 4 weeks of observation were clustered into four patterns of adherence. A group of patients started with good adherence and ended with poor adherence (slope < - 0.05), Figure 3.9(a). One group demonstrated poor adherence throughout and another group had good adherence throughout (slope between -0.05 and 0.05), Figure 3.9(b) and 3.9(c). The last group had a significant improvement in adherence to approximately 80% (slope > 0.05), Figure 3.9(d).

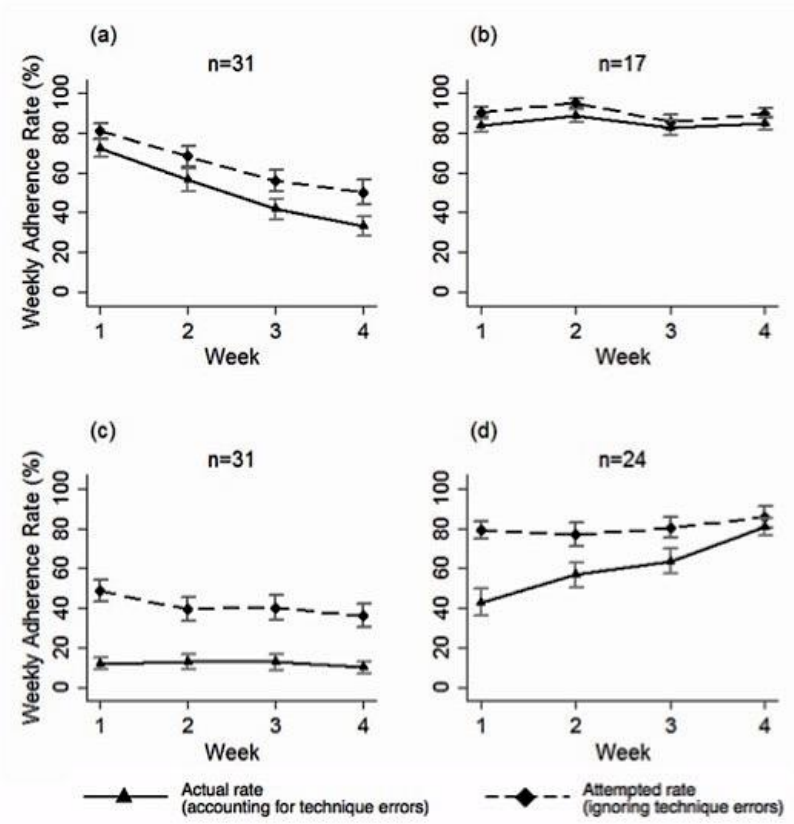

Figure 3.9: Variation in inhaler use over time. (a) Good initial adherence followed by poor adherence; (b) good adherence throughout study; (c) poor adherence throughout study; and (d) poor initial adherence followed by good adherence. Attempted rate represents the rate determined from the number of audio files retrieved from the INCA^{TM} device. Actual rate represents the attempted rate minus the rate of technique errors.

3.1.5. Determinants of adherence

There were no statistically significant relationships between adherence rates and age, gender, smoking history, GP use, diagnosis, exacerbations or hospitalizations. Lower technique error rate was associated with higher socio-economic class and private health insurance ($p<0.01$), while patients with secondary level education demonstrated better combined adherence ($p=0.015$).

3.2. Discussion

Practice guidelines suggest addressing aspects of inhaler adherence and technique prior to escalation of drug therapy.(13) The INCA™ device was applied in a community care setting to investigate the prevalence of technique and temporal non-adherence to inhaler therapy and to identify the most common technique errors. The findings show that errors in both over/under-use, multiple doses and errors in inhaler handling are equally common.

Failure to achieve a sufficient inhalation flow rate, drug blistering with no subsequent inhalation, exhalation of humid air into the dry powder inhaler and rapid exhalation after inhalation constituted 21% of all events and were the most common technique errors, often in combination. However, technique errors were not present in all inhalations from the same individual and were interspersed with events demonstrating correct technique, highlighting the day-to-day variation in inhaler use in this setting.

Temporal adherence was also variable. Only 41 patients had good temporal adherence (adherence rate > 80%) throughout the study period. A significant proportion (one third) had a decline in their rate of adherence over time, which

may have been due to a ‘Hawthorne effect’, that is, as patients received the device they were initially adherent to the medication, and this declined over time. Another group had improved adherence over the study period over time. Finally, two more groups had relatively constant adherence; one group with good adherence and one with poor adherence. These variations have been difficult to capture with previous measures of adherence since all prior methods involve averaging of doses over a period of time. It is clear that inhaler adherence changes with time, likely in response to underlying disease or social factors. Only by monitoring these trends, can inhaled therapy be personalized for individual patients.

This study has several limitations. The setting in which this study was performed (community care) may limit the generalizability of the findings to clinical research or secondary/ tertiary care settings. Nevertheless, we were able to describe adherence in a large group of patients, who represent the most common users of inhalers. The lack of a significant relationship between variables such as age, disease severity or diagnosis and adherence may be due to the small sample size and lack of statistical power. Future studies should specifically assess adherence in different age groups, different disease severities, different socio-economic classes and education histories. In order to explore this, longer, larger and intervention based studies are currently underway in both asthma and COPD using the INCA^{TM} device. Finally, this study did not assess symptoms or measures of disease control, since a longer follow-up period exceeding 6 months is required.

CHAPTER 4: The effect of inhalation parameters on delivered dose and an acoustic method to quantify this effect

4.1. The relationship between baseline spirometric PIFR/ IC and Diskus™ inhalation parameters: Is baseline spirometry sufficient for estimating peak flow from a Diskus™ DPI?

4.1.1. Results

Table 4.1 shows the demographics and baseline lung function parameters for the study subjects. Approximately two thirds of the recruited patients had a diagnosis of obstructive airways disease (32% asthma and 32% COPD). Twenty seven percent were either healthy or had a non-respiratory condition (healthy/NRC). The asthma and COPD groups were, on average, older than the healthy/ NRC group ($p=0.001$ and $p<0.001$, respectively). The COPD group was also significantly older than the asthma group ($p<0.001$). Gender and BMI differences among groups were not significant.

Table 4.1: Demographics and baseline lung function tests for patients by disease category.

	All	Asthma	COPD	NMD	Healthy/ NRC
Number	85	27	27	8	23
Age (years)	51.8±17.8 (18-80)	52.6±15.9 (18-76)	66.0±8.4 (44-80)	41.9±19.6 (18-78)	37.8 ± 14.6 (20-65)
Gender (M:F%)	42:58	30:70	37:63	75:25	52:48
BMI (kg/m^2)	27.6±6.8 (16.7-49.2)	27.0±6.0 (16.7-37.8)	26.2±5.3 (18.0-38.0)	29.0±8.6 (21.3-48.2)	29.5±8.6 (19.5-49.2)
FEV_1 (L)	2.02±1.04 (0.24-5.07)	1.74±0.70 (0.93-3.38)	1.56±0.75 (0.24-3.05)	1.29±1.21 (0.33-3.06)	3.13±0.90 (1.58-5.07)
FVC (L)	2.75±1.12 (0.38-5.66)	2.45±0.78 (1.23-3.99)	2.41±0.79 (0.63-3.60)	1.69±1.69 (0.38-4.18)	3.83±1.02 (1.89-5.66)
FEV_1/ FVC	0.72±0.16 (0.35-0.99)	0.71±0.16 (0.44-0.94)	0.62±0.16 (0.35-0.85)	0.80±0.09 (0.71-0.90)	0.83±0.08 (0.70-0.99)

NRC: Non-respiratory condition; NMD: Neuromuscular disease

The FEV_1 and FVC were significantly lower in the asthma, COPD and neuromuscular disease (NMD) groups compared to the healthy/ NRC controls ($p<0.002$ for all comparisons). As expected, asthmatics and COPD patients had a lower FEV_1/FVC ratio compared to both the NMD and healthy/ NRC groups.

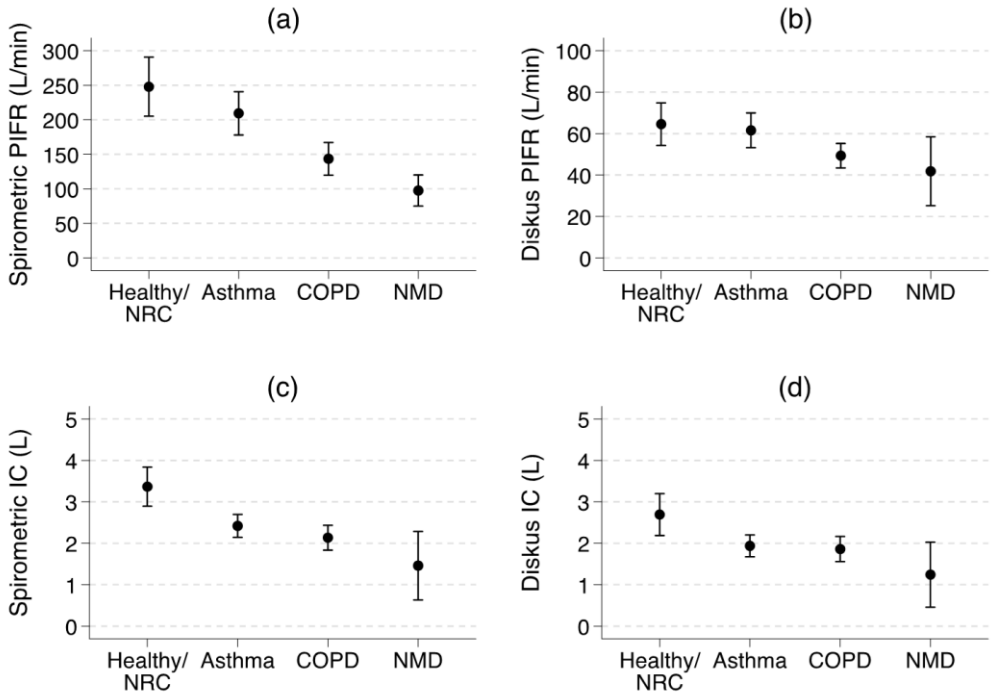

Figure 4.1: Mean and 95% Confidence Interval plots for (a) spirometric PIFR versus patient disease group; (b) Diskus^{TM} PIFR versus patient disease group; (c) spirometric IC versus patient disease group; and (d) Diskus^{TM} IC versus patient disease group. Healthy/ non-respiratory condition (NRC): n=23, asthma: n=27, chronic obstructive pulmonary disease (COPD): n=27, neuromuscular disease (NMD): n=8.

Figure 4.1 shows the mean spirometric PIFR and 95% confidence interval for the 4 groups of patients studied. The mean spirometric PIFR was significantly lower for the COPD and NMD groups compared to the asthma or healthy/ NRC groups ($p \leq 0.001$, see Tables 4.2 and 4.3). These trends were also seen for mean Diskus^{TM} PIFR values (Figure 4.1(b) and Table 4.2). The mean spirometric PIFR for the NMD group was significantly lower than that for the COPD group ($p=0.005$, see Tables 4.2 and 4.3). The Diskus^{TM} PIFR for the COPD and NMD groups was more than 10 l/min lower than the healthy or asthma groups and was significantly lower than 60 l/min ($p<0.05$, see Tables 4.2 and 4.3). The proportions of patients in each group with a Diskus^{TM} PIFR < 60 l/min (the threshold for optimum drug delivery from the Diskus^{TM} inhaler) were significantly different (Table 4.4).

Table 4.2: Mean values for spirometric and Diskus^{TM} PIFR or IC according to patient disease group.

	Diskus^{TM} PIFR	**Spirometric PIFR**	**Diskus^{TM} IC**	**Spirometric IC**
Healthy/ NRC	64.57±25.12 (20-97)	247.87±104.35 (59-456)	2.69±1.24 (0.27-4.92)	3.37±1.16 (1.02-5.42)
Asthma	61.56±22.15 (17-102)	209.41±83.26 (59-415)	1.94±0.70 (0.60-3.37)	2.42±0.73 (1.17-3.78)
COPD	49.37±15.68 (22-83)	143.46±62.98 (55-275)	1.86±0.80 (0.36-3.34)	2.13±0.79 (0.71-3.59)
NMD	41.83±24.03 (10-88)	97.53±33.54 (55-153)	1.24±1.13 (0.33-3.87)	1.46±1.19 (0.42-4.13)

NRC: Non-respiratory condition; NMD: Neuromuscular disease

Table 4.3: p values for one-sided independent samples t-tests for comparisons of spirometric and Diskus™ PIFR between patient disease groups.(s) represents spirometric values and (d) represents Diskus™ values.

Peak Inspiratory Flow Rate (PIFR)				
	Healthy/ NRC	**Asthma**	**COPD**	**NMD**
Healthy/ NRC	--	NS *(d)*	0.009 *(d)*	0.019 *(d)*
Asthma	NS *(s)*	--	0.012 *(d)*	0.030 *(d)*
COPD	0.000 *(s)*	0.001 *(s)*	--	NS *(d)*
NMD	0.000 *(s)*	0.000 *(s)*	0.005 *(s)*	--

NS: not significant at an alpha of 0.05. NRC: Non-respiratory condition; NMD: Neuromuscular disease.

Table 4.4: Number of patients in each disease group with a Diskus™ PIFR greater than or equal to 60 l/min and less than 60 l/min. Results from Chi-squared test are shown. The null hypothesis is that the proportions of patients with a Diskus™ PIF value less than or equal to sixty is independent of their diagnosis. As the test statistic is greater than the critical value, we can reject the null hypothesis.

PIFR (l/min)	**Healthy/ NRC**	**Asthma**	**COPD**	**NMD**	**Totals**
≥ 60	14	15	8	1	38
<60	9	12	19	7	47
Totals	23	27	27	8	85

Chi-squared Statistic= 8.04; Critical Value= 7.815; df= 3. NRC: Non-respiratory condition; NMD: Neuromuscular disease.

The mean spirometric and Diskus^{TM} IC values with 95% CIs and the p-values for differences between group means are shown in Figures 4.1(c) and 4.1(d) and Tables 4.2 and 4.5, respectively. The mean spirometric and Diskus^{TM} IC of the healthy/NRC group was significantly (> 0.75 L) higher than the mean for the other three groups ($p \leq 0.001$ for spirometric PIFR; $p=0.004$ for Diskus^{TM} PIFR). The spirometric IC was also higher for the asthma and COPD groups compared to the NMD group; the differences were not statistically significant except for the spirometric IC from the asthma group compared to the NMD group ($p=0.03$).

Table 4.5: p values for one-sided independent samples t-tests for comparisons of spirometric and Diskus^{TM} IC between patient disease groups.(s) represents spirometric values and (d) represents Diskus^{TM} values.

Inspiratory Capacity (IC)				
	Healthy/ NRC	**Asthma**	**COPD**	**NMD**
Healthy/ NRC	--	0.004 *(d)*	0.004 *(d)*	0.004 *(d)*
Asthma	0.001 *(s)*	--	NS *(d)*	NS *(d)*
COPD	0.000 *(s)*	NS *(s)*	--	NS *(d)*
NMD	0.001 *(s)*	0.030 *(s)*	NS *(s)*	--

NS: not significant at an alpha of 0.05. NRC: Non-respiratory condition; NMD: Neuromuscular disease.

The p-values for two-sided independent samples t-tests for age groups, BMI groups and gender groups are shown in Table 4.6. The mean values for all four measured flow rate and volume parameters were significantly higher in younger patients (age <50 years). There was no difference between the obese (BMI >30 kg/m^2) versus non-obese groups or the male versus female groups.

Table 4.6: Mean differences and p values for two-sided independent samples t-tests for comparisons of spirometric or Diskus™ PIFR or IC across age groups, gender or BMI groups.

	Age (<50 vs >=50)	**Male vs Female**	**BMI (<30 vs >=30)**
Diskus™ PIFR	9.61 (0.025)	-0.429 (NS)	3.609 (NS)
Baseline PIFR	48.33 (0.017)	23.543 (NS)	-2.082 (NS)
Diskus™ IC	0.4803 (0.030)	0.2187 (NS)	0.1492 (NS)
Baseline IC	0.7755 (0.003)	0.8289 (NS)	0.0646 (NS)

NS: not significant at an alpha of 0.05.

The stepwise deletion regression showed that gender, height, weight, BMI, FEV_1, FVC and FEV_1/FVC were not significantly correlated with Diskus™ PIFR at a significance level of 0.05. Diskus™ PIFR was moderately correlated with spirometric PIFR and age (adjusted $R^2 = 0.58$, $p < 0.0001$) and the relationship is described by the following equation:

$$PIFR_{Diskus} = 0.139498 * PIFR_{Spirometric} - 0.2570845 * Age + 47.696 \quad (3.1)$$

$$PIFR_{Spirometric}: p < 0.0001, \eta^2 = 0.33661; Age: p = 0.019, \eta^2 = 0.03652$$

While Diskus™ PIFR from COPD and NMD patients was significantly different to that from healthy patients and asthmatics, this effect was modified by both age and spirometric PIFR in the stepwise regression and as a result, condition was no longer significant in the model. A scatterplot of Diskus™ PIFR versus spirometric PIFR with the line of best fit are shown in Figure 4.2.

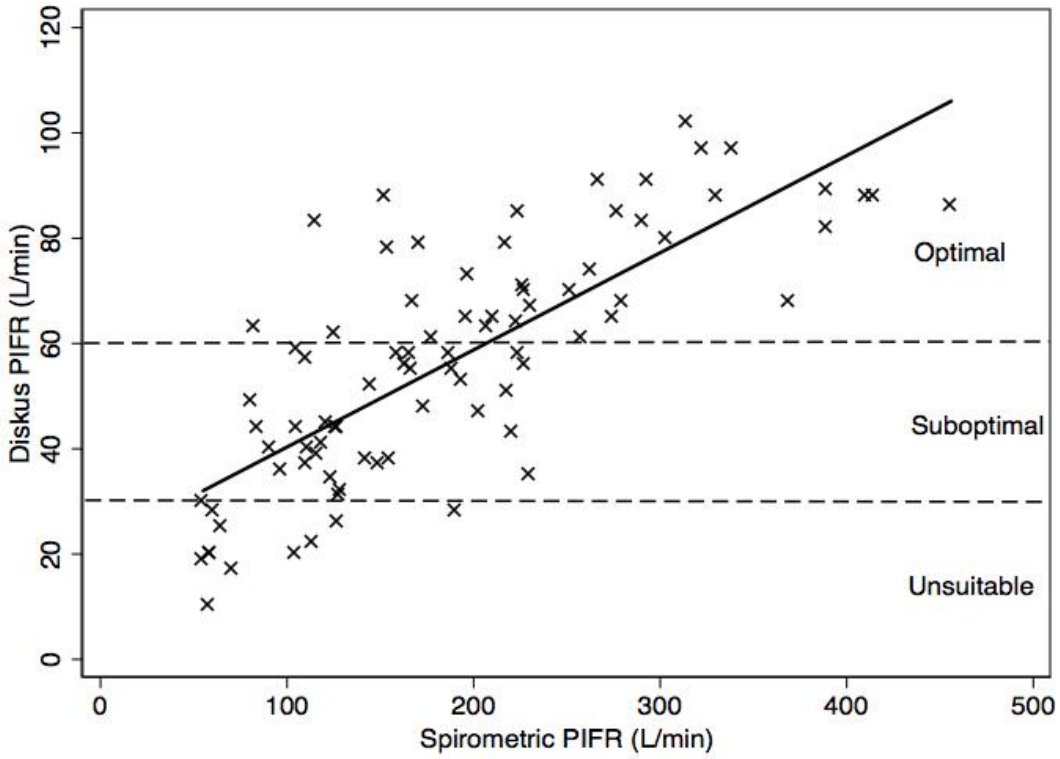

Figure 4.2: Scatterplot of Diskus™ PIFR versus spirometric PIFR showing line of best fit. Dashed lines represent Diskus™ PIFR of 30 l/min (minimum required) and 60 l/min (optimal PIFR for drug delivery). The Diskus™ dry powder inhaler is unsuitable in patients with a Diskus™ PIFR less than 30 l/min.

Diskus™ PIFR was binned according to a threshold of 60 l/min and a receiver-operating characteristic (ROC) curve of Spirometric PIFR versus Diskus™ PIFR category had an area under the curve of 0.89. At a spirometric PIFR cutoff of 196 l/min, 84% of Diskus™ PIFR values were correctly classified as either greater than or equal to 60 l/min or less than 60 l/min (sensitivity of 79% and specificity of 87%). When the Diskus™ PIFR was binned according to a threshold of 30 l/min, a spirometric cutoff of 115 l/min had a sensitivity of 86% and a specificity of 83% (86% correctly classified). The ROC Curves for a Diskus™ threshold of 60 l/min and 30 l/min are shown in Figures 4.3 and 4.4, respectively.

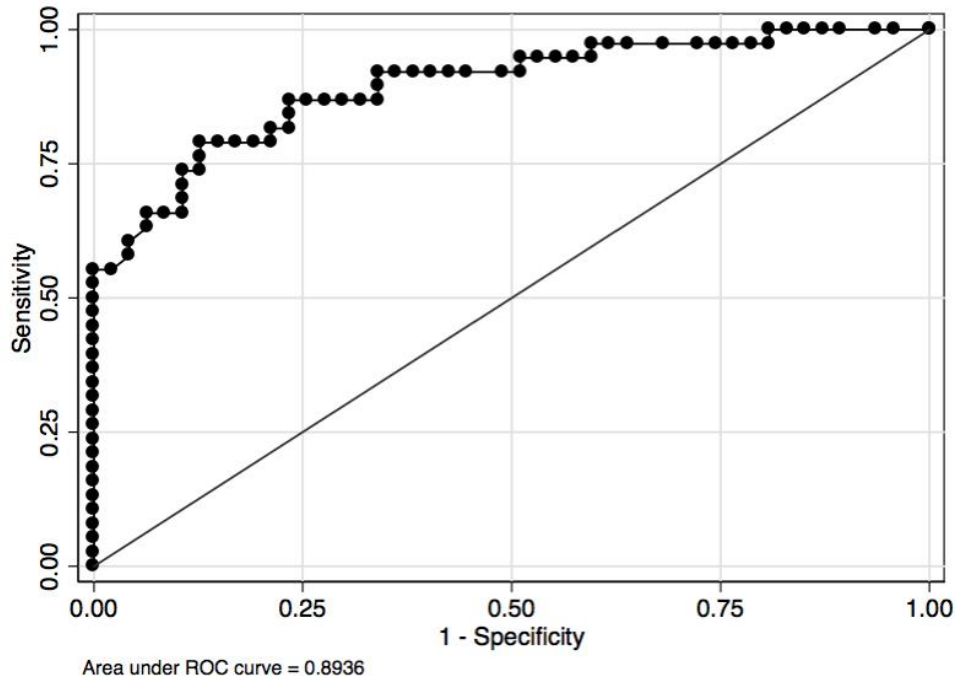

Figure 4.3: Receiver operating characteristic curve for spirometric PIFR versus binary Diskus™ PIFR based on threshold of 60 l/min. The solid line represents an AUC of 0.5.

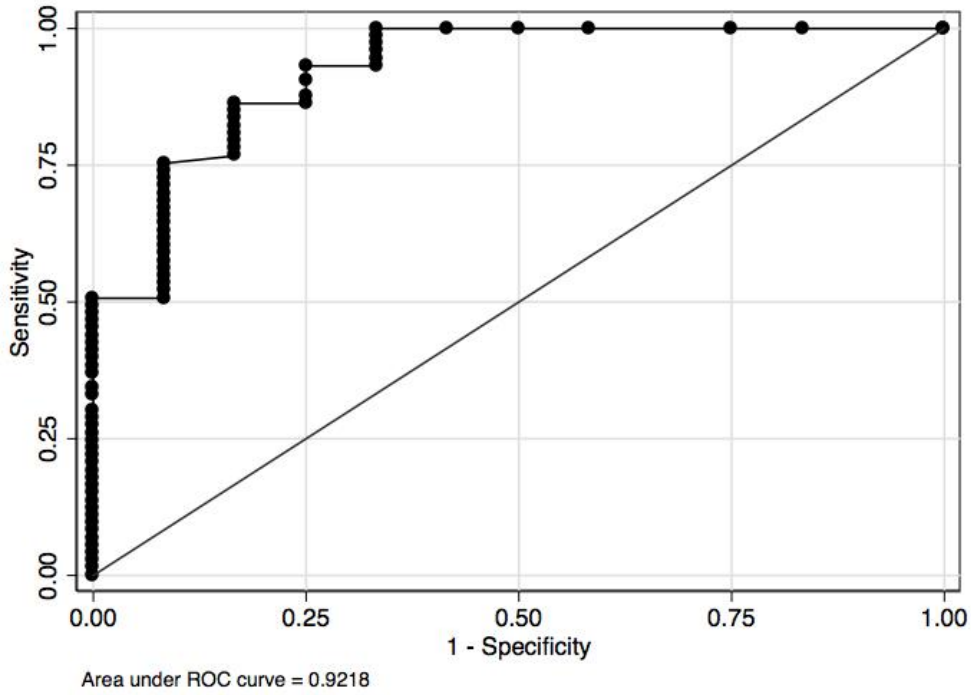

Figure 4.4: Receiver operating characteristic curve for spirometric PIFR versus binary Diskus™ PIFR based on threshold of 30 l/min. The solid line represents an AUC of 0.5.

4.1.2. Discussion

The purpose of this study was to highlight the differences in inspiratory flow rates and volumes generated using a Diskus™ DPI based on patient demographics and underlying disease. Our results show that patients with COPD and neuromuscular disease do not generate as high a PIFR (spirometric or through Diskus™) as healthy subjects, asthmatics or patients with non-respiratory conditions. Healthy subjects also have a significantly higher spirometric and Diskus™ IC compared to patients with respiratory-related diseases. Numerous authors have shown that drug delivery from a Diskus™ DPI is dependent on the PIFR of inhalation and that ideally, the PIFR should be above 60 l/min for optimum fine particle delivery. It is clear that the decision to start a patient on an inhaled medication delivered via a Diskus™ DPI should take into account the age of the patient and the underlying disease. It is likely that the differences seen between the PIFR of asthmatics versus COPD patients is explained by the fact that the COPD patients who were recruited for this study were older than the patients in the other groups. The lower PIFR in the patients with Neuromuscular disease is most likely explained by the pathophysiology of their underlying disease process leading to poor muscle function and contraction.

The second aim of our study was to determine whether spirometric PIFR measurements could be used to estimate whether patients would be suitable for the Diskus™ DPI based on PIFR criteria. There was a moderate correlation between spirometric and Diskus™ PIFR and the use of spirometric PIFR was very sensitive and specific for categorizing the Diskus™ PIFR as either greater than or equal to 60 l/min or less than 60 l/min.

Based on the stepwise deletion regression, underlying condition was not a significant variable in the model but it is likely that the differences in Diskus™ PIFR seen among diseases are directly related the differences in mean age and spirometric PIFR among groups. As expected, COPD patients were older than the other groups and had lower PIFRs than asthmatics and healthy

patients. Since these three groups were the largest in this study, it is clear why Diskus™ PIFR is also related to age. Age should therefore be taken into account when making a decision about suitability for a Diskus™ DPI.

It is very difficult to subjectively estimate a patient's inspiratory flow rate adequacy when using a checklist to evaluate inhaler technique. In the absence of a Clement-Clarke In-Check Dial™ for estimating Diskus™ PIFR, we believe that our method allows a much better estimation of the flow rate of inhalation from the Diskus™ than subjective assessment. Spirometric PIFR cutoffs of 196 l/min or 115 l/min correlate with a Diskus™ PIFR of 60 l/min (optimal delivery) and 30 l/min (minimum required for successful use), respectively. Our study showed that no patient with a spirometric PIFR above 196 l/min had a Diskus™ PIFR below 30 l/min. The 196 l/min spirometric cutoff is more useful in the general practice setting. It will identify all patients who are likely to have the minimal required Diskus™ PIFR of 30 l/min. Any patient with a spirometric PIFR below 196 l/min should have further testing possibly using the Clement Clark In-check Dial or consideration of an alternate device.

The use of spirometric PIFR can direct the clinician to use more sophisticated techniques, such as the INCA™ device, to train a patient and monitor his/ her technique longitudinally. It is noteworthy that our method for estimation of Diskus™ PIFR is not perfect (we could not explain about 40% of the variance seen in Diskus™ PIFR by using spirometric PIFR). PIFR is a very effort-dependent measure and variations in effort exerted by the patient could explain the differences seen in spirometric and Diskus™ PIFR values. It is also likely that a patient's inspiratory effort changes when they are acutely unwell. Therefore, while use of spirometric PIFR is a suitable substitute for the Clement Clark In-Check Dial for once off assessments of Diskus™ inhaler technique in a clinic setting, a method of longitudinally monitoring Diskus™ PIFR will be more beneficial.

4.2. Developing an acoustic method of estimating inspiratory flow rate and volume from an inhaler

4.2.1. Results

Table 4.7 shows the demographics and baseline lung function of the 15 healthy volunteers enrolled in this study. The ethnic origin of subjects was Caucasian for 93.3% (14/15) and Hispanic for the remaining 6.7% (1/15). All subjects had an FEV1/FVC ratio >0.7 and a predicted FEV1 > 89%, confirming normal baseline lung function according to ATS standards.

Table 4.7: Summary of demographics and baseline lung function data from all subjects (n=15).

Variable	Mean	SD	Range
Age (years)	25.9	4.2	22-35
Gender (M:F)	(9:6)		
Height (cm)	174.5	6.4	164-185
Weight (kg)	72.8	9.0	56-91
BMI[a] (kg/m^2)	23.86	2.21	20.8-29.7
FEV1[b] (L)	3.98	0.58	2.79-4.85
FEV1[b] (%) Predicted	99.33	5.33	92-110
FVC[c] (L)	4.90	0.73	3.41-6.24
FEV1/FVC Ratio	0.81	0.06	0.70-0.91
PEFR[d] (l/min)	547.6	103.7	384-744
FIVC[e] (L)	4.56	0.67	3.34-5.76
PIFR[f] (l/min)	402.1	82.1	276-535

[a] BMI – Body Mass Index
[b] FEV1 – Forced Expiratory Volume in 1 second
[c] FVC – Forced Vital Capacity
[d] PEFR – Peak Expiratory Flow Rate
[e] FIVC – Forced Inspiratory Vital Capacity
[f] PIFR – Peak Inspiratory Flow Rate

A total of 120 audio files were obtained from the 15 subjects. 17 audio files were discarded due to an INCA^{TM} device formatting error. In this study the PIFR range of interest was between 0-100 L/Min and a subsequent 17 audio files were omitted that had PIFR values greater than 100 L/Min, leaving a total of 86 observations from the 15 subjects. For each inhalation the spirometer provided values for PIFR and IC. PIFR was compared to MA, MAD and RMS of the inhalation signal, while P_{ave} at several select frequency bands (described earlier) was also compared to PIFR.

It was found that MA, MAD and RMS were all highly correlated with PIFR ($p<0.0001$) at a significance level of $\alpha = 0.05$. The coefficients of determination were found to be $R^2 = 0.8386$ for MA, $R^2 = 0.8340$ for MAD and $R^2 = 0.8320$ for RMS. P_{ave} for a range of select frequency bands was also calculated. Using a GLS regression model to compare PIFR to P_{ave} it was found that the relationship was also highly correlated for all of the frequency bands ($p<0.0001$, $\alpha = 0.05$). It is worth noting that at higher powers, the GLS regression model will give PIFRs exceeding the maximum possible flow rate through the inhaler. The P_{ave} in the frequency band 300-600 Hz had the strongest correlation with PIFR, as the GLS regression model for this frequency band had an R^2 value of 0.9079. A complete analysis of the relationship between P_{ave} and PIFR for each of the frequency bands analysed is presented in Table 4.8. The overall results demonstrating the relationship between MA, MAD, RMS, P_{ave} and PIFR can be seen in Figure 4.5. Individual plots of acoustic parameters versus PIFR for each subject are shown in Figure 4.6 – 4.9 and the associated GLS regression outputs can be found in Figures 4.10 – 4.13.

Table 4.8: Correlation scores between P_{ave} and PIFR.

Frequency Band (Hz)	Coefficient of Determination (R^2)
20-40	0.7865
40-70	0.7018
70-150	0.8067
150-300	0.8461
300-600	0.9079
70-300	0.8427
70-450	0.8746
100-300	0.8431
100-450	0.7018
150-450	0.8807

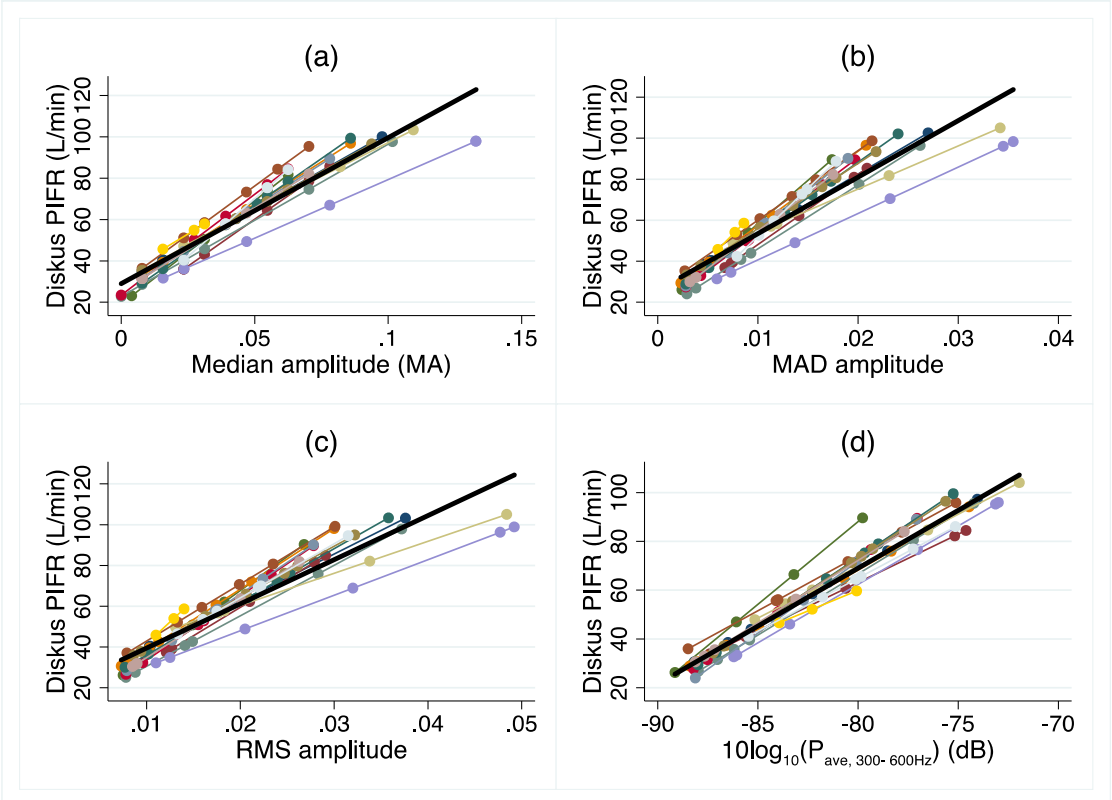

Figure 4.5: PIFR versus (a) MA, (b) MAD amplitude, (c) RMS amplitude and (d) average power (P_{ave}) in the frequency band 300-600Hz. Plotted points are calculated PIFRs based on regression equation for each subject. Black line represents overall regression model equation.

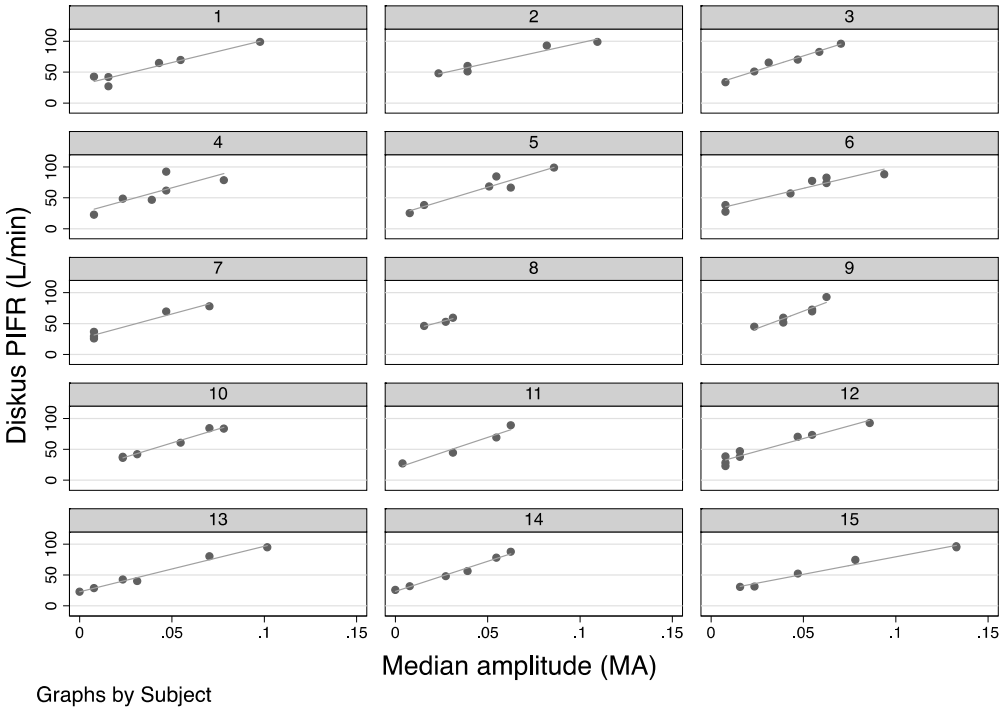

Figure 4.6: Measured Diskus^{TM} PIFR versus median amplitude for each subject with linear trendline.

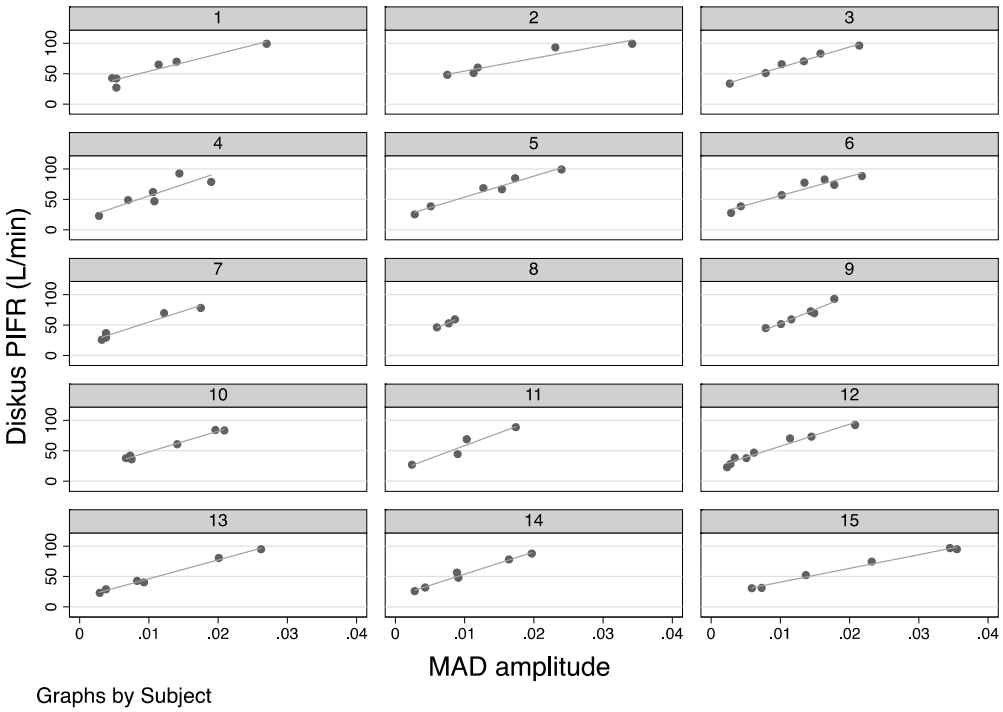

Figure 4.7: Measured Diskus^{TM} PIFR versus mean absolute deviation (MAD) of amplitude for each subject with linear trendline.

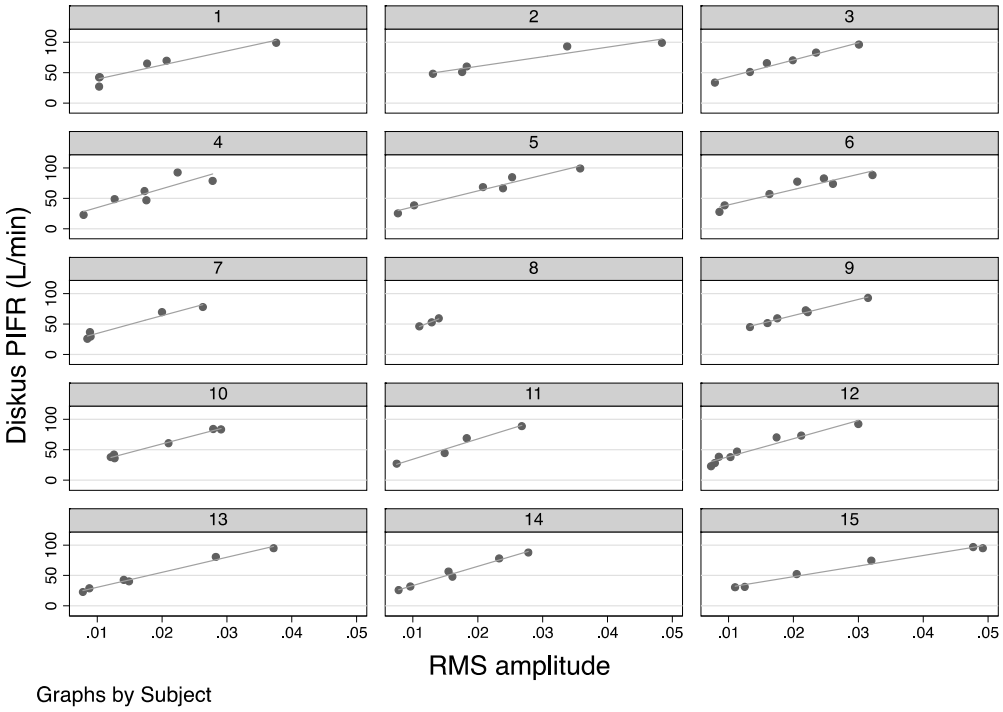

Figure 4.8: Measured Diskus^{TM} PIFR versus root mean square (RMS) of amplitude for each subject with linear trendline.

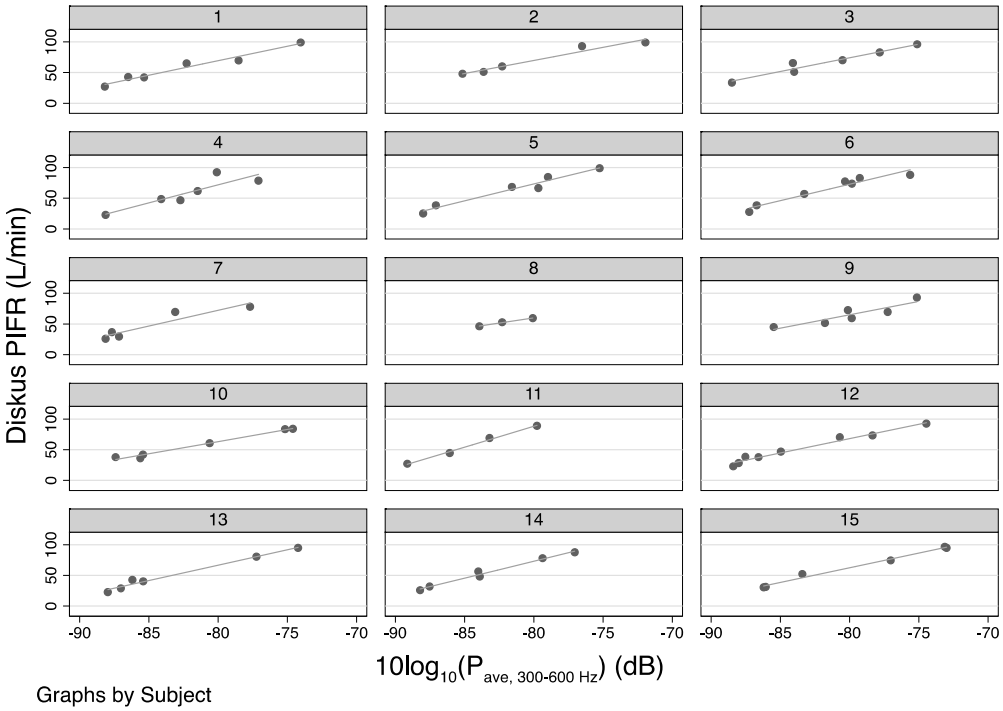

Figure 4.9: Measured Diskus^{TM} PIFR versus average power (P_{ave}) in 300-600 Hz frequency band for each subject with linear trendline.

```
Random-effects GLS regression                   Number of obs      =        86
Group variable: Subject                         Number of groups   =        15

R-sq:  within  = 0.8949                         Obs per group: min =         3
       between = 0.5236                                        avg =       5.7
       overall = 0.8386                                        max =         8

                                                Wald chi2(1)       =    528.38
corr(u_i, X)   = 0 (assumed)                    Prob > chi2        =    0.0000
```

PIFR	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
MA	728.662	31.69965	22.99	0.000	666.5318	790.7921
_cons	28.11024	1.839411	15.28	0.000	24.50506	31.71542
sigma_u	3.1121892					
sigma_e	8.0158992					
rho	.1309937	(fraction of variance due to u_i)				

Figure 4.10: GLS regression results for measured Diskus™ PIFR vs Median amplitude.

```
Random-effects GLS regression                   Number of obs      =        86
Group variable: Subject                         Number of groups   =        15

R-sq:  within  = 0.9038                         Obs per group: min =         3
       between = 0.5306                                        avg =       5.7
       overall = 0.8340                                        max =         8

                                                Wald chi2(1)       =    542.60
corr(u_i, X)   = 0 (assumed)                    Prob > chi2        =    0.0000
```

PIFR	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
MAD	2879.436	123.6142	23.29	0.000	2637.156	3121.715
_cons	24.45219	1.963524	12.45	0.000	20.60375	28.30062
sigma_u	3.2183084					
sigma_e	7.6725414					
rho	.14962006	(fraction of variance due to u_i)				

Figure 4.11: GLS regression results for measured Diskus™ PIFR vs mean absolute deviation (MAD) of amplitude.

```
Random-effects GLS regression                   Number of obs      =        86
Group variable: Subject                         Number of groups   =        15

R-sq:  within  = 0.8958                         Obs per group: min =         3
       between = 0.5478                                        avg =       5.7
       overall = 0.8320                                        max =         8

                                                Wald chi2(1)       =    504.22
corr(u_i, X)   = 0 (assumed)                    Prob > chi2        =    0.0000
```

PIFR	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
RMS	2243.905	99.92989	22.45	0.000	2048.046	2439.764
_cons	16.35855	2.273995	7.19	0.000	11.9016	20.8155
sigma_u	2.9476972					
sigma_e	7.9830894					
rho	.11998181	(fraction of variance due to u_i)				

Figure 4.12: GLS regression results for measured Diskus™ PIFR vs root mean square (RMS) of amplitude.

```
Random-effects GLS regression                   Number of obs      =        86
Group variable: Subject                         Number of groups   =        15

R-sq:  within  = 0.9338                         Obs per group: min =         3
       between = 0.6329                                        avg =       5.7
       overall = 0.9078                                        max =         8

                                                Wald chi2(1)       =    976.50
corr(u_i, X)   = 0 (assumed)                    Prob > chi2        =    0.0000
```

PIFR	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
Power	4.811537	.1539739	31.25	0.000	4.509753	5.11332
_cons	454.0713	12.692	35.78	0.000	429.1954	478.9471
sigma_u	3.0188003					
sigma_e	6.3644363					
rho	.18366185	(fraction of variance due to u_i)				

Figure 4.13: GLS regression results for measured Diskus™ PIFR vs average power (P_{ave}) in 300-600 Hz frequency band.

With the analysis of MA, MAD, RMS and P_{ave} it is possible to estimate IC. Figure 4.5 demonstrates that it is possible to estimate values for PIFR from analysis of the inhalation signal. IC can subsequently be calculated by using equation 2.3.

GLS regression demonstrated that IC can be estimated using MA, MAD, RMS and P_{ave} ($P<0.001$, $\alpha = 0.05$). The coefficients of determination (R^2) for predicting IC were 0.9020 for MA, 0.9047 for MAD, 0.8989 for RMS and 0.9245 for P_{ave} in the frequency band 300-600 Hz. Figure 4.13 presents plots of measured Diskus^{TM} IC versus IC estimated from MA (IC-MA), MAD (IC-MAD), RMS (IC-RMS) and P_{ave} (IC-P_{ave}). Individual plots of calculated versus measured IC for each subject are shown in Figures 4.14 – 4.17 and GLS regression outputs for each acoustic parameter are in Figures 4.18 – 4.21.

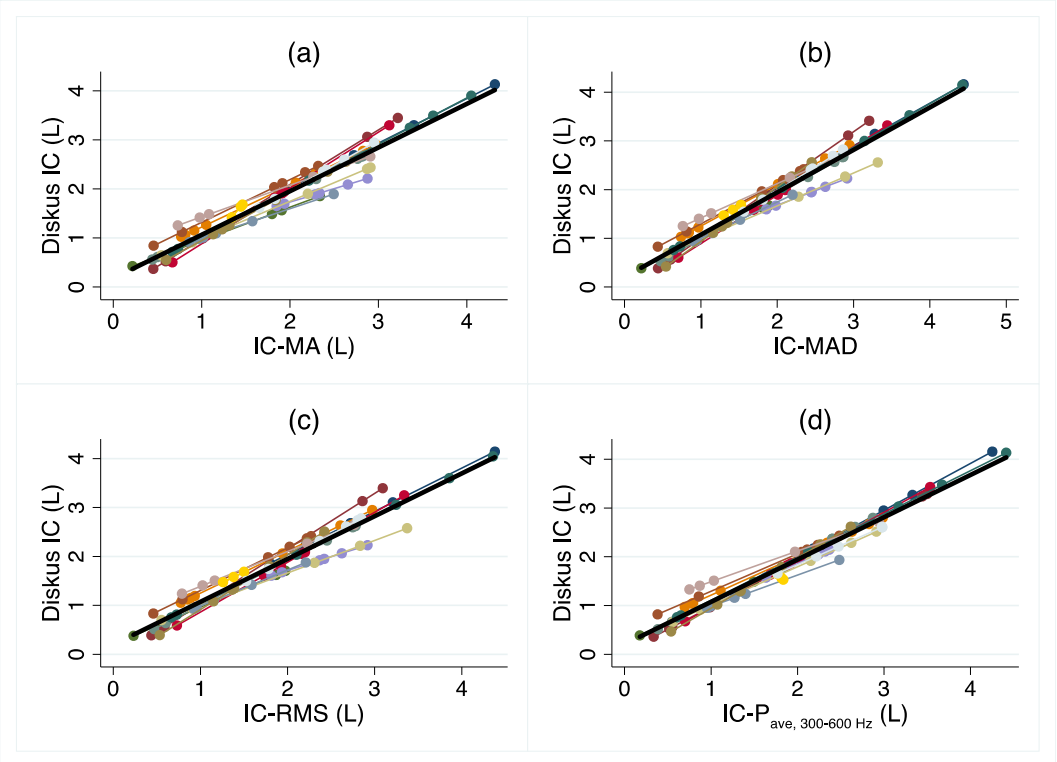

Figure 4.13: Measured IC versus IC calculated from (a) MA, (b) MAD, (c) RMS and (d) P_{ave} in 300-600 Hz frequency band. Plotted points are calculated ICs based on regression equation for each subject. Black line represents overall regression model equation.

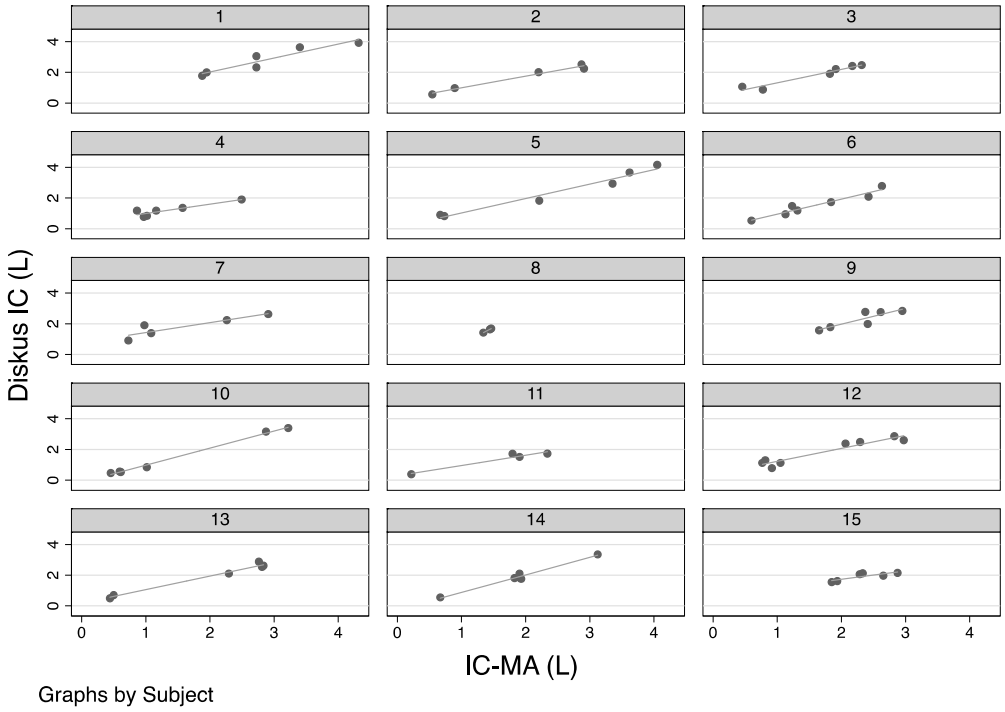

Figure 4.14: Measured Diskus^{TM} IC versus IC calculated from median amplitude (MA) for each subject with linear trendline.

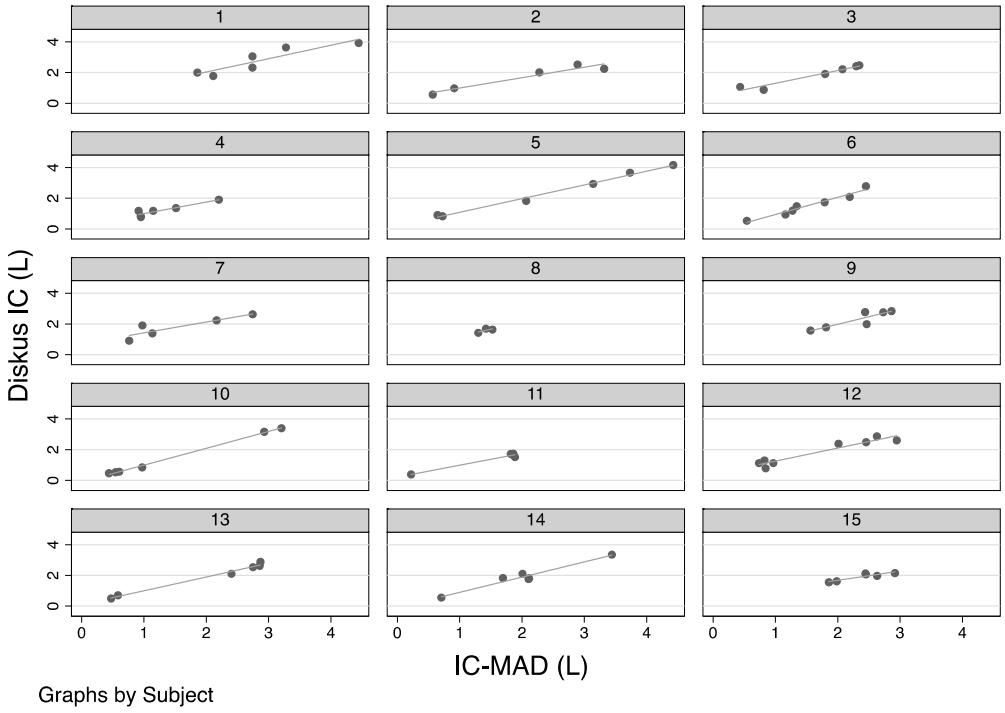

Figure 4.15: Measured Diskus^{TM} IC versus IC calculated from mean absolute deviation (MAD) amplitude for each subject with linear trendline.

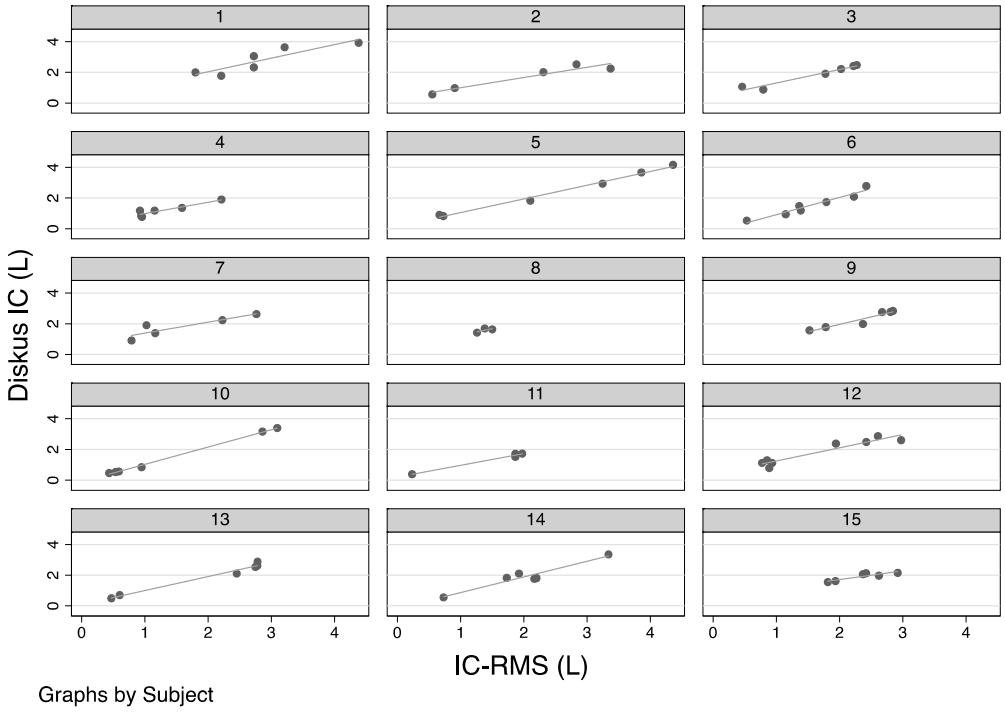

Figure 4.16: Measured Diskus^{TM} IC versus IC calculated from root mean square (RMS) amplitude for each subject with linear trendline.

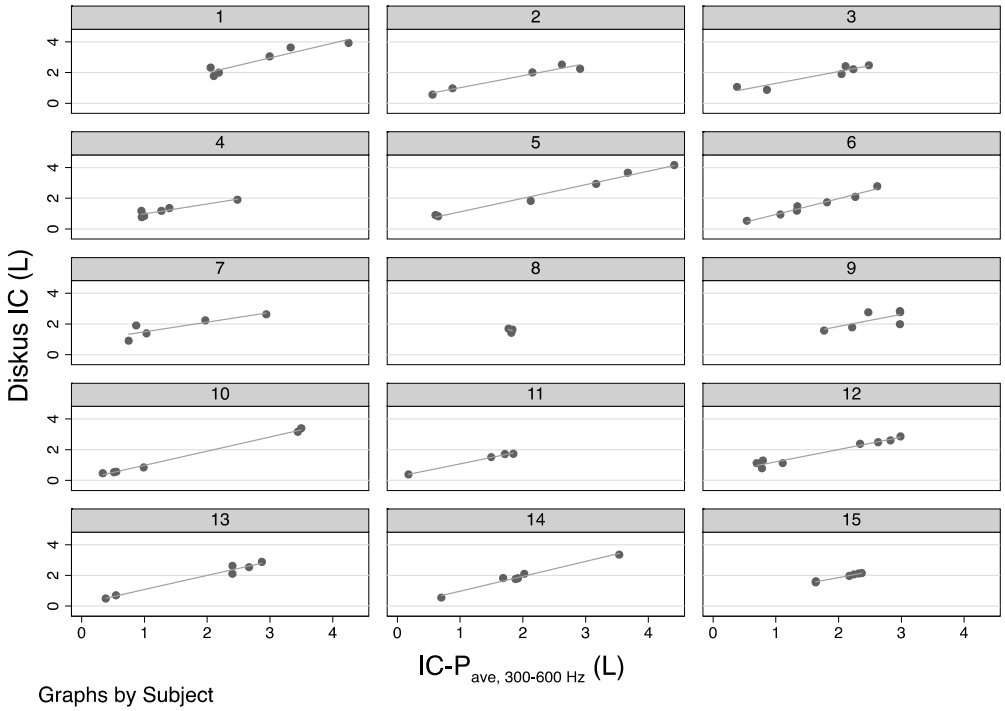

Figure 4.17: Measured Diskus^{TM} IC versus IC calculated from average power (Pave) in 300-600 Hz frequency band for each subject with linear trendline.

```
Random-effects GLS regression                   Number of obs      =        86
Group variable: Subject                         Number of groups   =        15

R-sq:  within  = 0.9176                         Obs per group: min =         3
       between = 0.8343                                        avg =       5.7
       overall = 0.9020                                        max =         8

                                                Wald chi2(1)       =    856.59
corr(u_i, X)   = 0 (assumed)                    Prob > chi2        =    0.0000
```

FIVC	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
ICc_MA	.8933534	.0305236	29.27	0.000	.8335282	.9531785
_cons	.166087	.072242	2.30	0.022	.0244953	.3076788
sigma_u	.13698415					
sigma_e	.24830767					
rho	.23332929	(fraction of variance due to u_i)				

Figure 4.18: GLS regression results for measured Diskus™ IC vs IC calculated from median amplitude.

```
Random-effects GLS regression                   Number of obs      =        86
Group variable: Subject                         Number of groups   =        15

R-sq:  within  = 0.9245                         Obs per group: min =         3
       between = 0.8305                                        avg =       5.7
       overall = 0.9047                                        max =         8

                                                Wald chi2(1)       =    924.84
corr(u_i, X)   = 0 (assumed)                    Prob > chi2        =    0.0000
```

FIVC	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
ICc_MAD	.8817194	.0289933	30.41	0.000	.8248936	.9385451
_cons	.1808967	.070627	2.56	0.010	.0424703	.3193231
sigma_u	.1428549					
sigma_e	.23765254					
rho	.26542486	(fraction of variance due to u_i)				

Figure 4.19: GLS regression results for measured Diskus™ IC vs IC calculated from mean absolute deviation (MAD) of amplitude.

```
Random-effects GLS regression                   Number of obs      =        86
Group variable: Subject                         Number of groups   =        15

R-sq:  within  = 0.9172                         Obs per group: min =         3
       between = 0.8287                                        avg =       5.7
       overall = 0.8989                                        max =         8

                                                Wald chi2(1)       =    842.30
corr(u_i, X)   = 0 (assumed)                    Prob > chi2        =    0.0000
```

FIVC	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
ICc_RMS	.8864277	.0305429	29.02	0.000	.8265647	.9462907
_cons	.1727882	.0730144	2.37	0.018	.0296826	.3158939
sigma_u	.14047185					
sigma_e	.24887593					
rho	.24160601	(fraction of variance due to u_i)				

Figure 4.20: GLS regression results for measured Diskus™ IC vs IC calculated from root mean square (RMS) of amplitude.

```
Random-effects GLS regression                   Number of obs      =        86
Group variable: Subject                         Number of groups   =        15

R-sq:  within  = 0.9305                         Obs per group: min =         3
       between = 0.8917                                        avg =       5.7
       overall = 0.9244                                        max =         8

                                                Wald chi2(1)       =   1063.98
corr(u_i, X)   = 0 (assumed)                    Prob > chi2        =    0.0000
```

FIVC	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
ICc_Power	.8666558	.0265693	32.62	0.000	.8145808	.9187307
_cons	.2064111	.0614334	3.36	0.001	.0860039	.3268183
sigma_u	.10058063					
sigma_e	.22807468					
rho	.1628155	(fraction of variance due to u_i)				

Figure 4.21: GLS regression results for measured Diskus™ IC vs IC calculated from average power (P_{ave}) in 300-600 Hz frequency band.

4.2.2. Discussion

The aim of this study was to investigate whether acoustic features of inhalations could be used to estimate PIFR and IC in 15 healthy subjects. The main results reveal that MA, MAD and RMS of the amplitude and P_{ave} at a range of different frequency bands all provided a robust method of estimating PIFR and IC. The high level of correlation between PIFR and IC from the acoustic measurements to the ‘‘gold standard’’ method using spirometry is a promising result, suggesting that this approach may be used in future validation studies.

Several previous studies have investigated the relationship between respiratory sounds and airflow. Unlike earlier studies, which have investigated respiratory sounds recorded on the chest wall and trachea, this study focused on sounds generated during inhaler use. Inhaler sounds are a mixture of both respiratory sounds and sounds from the inhaler itself. The microphone was located in the INCA^{TM} device, which was securely bonded to the inhaler in a location less than 5cm from the mouth. The results of this study are in accordance with previous research which established that variations in flow are reflected in the intensity and frequency distribution of the sounds generated.(160, 161) A study by Hossain and Moussavi indicated that P_{ave} had the strongest correlation with flow rate from respiratory sounds. The results of the present study found that P_{ave} had the strongest correlation with flow rate from inhaler sounds.(146) The study also reported that the optimum frequency band to calculate P_{ave} was 150-450 Hz for healthy subjects, while in the present study we found this optimum frequency band to be 300-600 Hz for inhaler sounds. It is therefore clear to see that the sounds created by the inhaler are different in comparison to normal respiratory sounds. Inhaling through the narrow opening of the Diskus^{TM} inhaler has created a shift in sound intensity towards higher frequencies.

The additional dead space volume of the airtight container adds additional resistance to the overall pathway of the spirometer. This means that a slightly greater patient effort is required in order to obtain PIFR and IC values that

would have been reached without the airtight container. This could lead to values of MA, MAD, RMS and P_{ave} obtained being slightly higher than they should be for the corresponding PIFR and IC values. However, for the purposes of this study it was decided that the effects of the container's dead space is small enough to be negligible, given that the ranges studied were quite large (range of 100 l/min for PIFR and 3.54 L IC). The additional dead space of the container also met ATS 2005 requirements for spirometry, in that the total dead space of the circuit was less than 350 ml. One point to consider in this study also is that if the sound is generated by the flow through the inhaler, the frequency content of the sound may be proportionally shifted to higher frequencies at higher flows.

The current methods of assessing patients' inhaler technique are limited. At present clinicians make a subjective decision on whether a patient's inhalation is sufficiently adequate for their medication to reach their airways. However an effective inhalation is dependent on inspiratory flow rate, which cannot be measured subjectively. PIFR can be measured using a Clement Clarke *In-Check Dial*™ device,(162, 163) although this device is not widely used and when it is used, it is primarily in clinical environments. Additionally, the effort patients exert in front of the clinician may not correlate to the effort they put into using their inhaler on a day-to-day basis. The method we propose in this paper allows PIFR values from real world patient inhaler use to be acquired, in addition to IC values.

The objective of this study was to demonstrate the feasibility of using acoustic measurements to estimate PIFR/IC from inhalers. The regression models are inherently biased to the dataset used and hence cannot be used to estimate the 95% CI for a population of individuals. Nonetheless, the regression outputs show that the 95% CIs for the variables are actually relatively small, proving the potential of carrying out a validation study on a larger population. There are numerous potential clinical applications for a system that can accurately predict PIFR and IC from patients' inhalations during inhaler use. A standard threshold could be put in place to inform clinicians whether a patient performed an effective or ineffective inhalation. PIFR and IC could also be

monitored on a day-to-day basis, providing the opportunity to assess patients' respiratory condition over time. Monitoring PIFR and IC longitudinally may provide the opportunity to predict and prevent exacerbations before they take place. Analysis of PIFR may also show when narrowing of the airways occurs, while analysis of IC variations might be used to study dynamic hyperinflation, and monitor the drop in IC associated with exacerbations. Informing patients of their day-to-day PIFR and IC values may also encourage them to take better control of their respiratory disease, as they may come to realise that a greater effort is required on their part, in order to help deliver the medication to their airways. Such active feedback may provide the opportunity to improve the efficacy of the medication, reduce exacerbations and lower the frequency of admittance to hospital emergency departments.

4.3. Validation of an acoustic method for estimating inspiratory flow rate and volume from an inhaler using acoustic measurements in a respiratory disease cohort.

4.3.1. Results

Eighteen of the 110 patients recruited had corrupted audio recordings. Table 4.9 shows the baseline demographics and lung function for the remaining 92 patients. The majority of the patients had obstructive airways disease, either asthma or COPD. Asthmatics, obese patients and patients with non-respiratory conditions had a significantly higher PIFR than the other patient groups.

Table 4.9: Demographics and baseline lung function tests for patients by disease category.

	All	Asthma	COPD	NMD	Obesity	ORC	NRC
Number	92	27	25	9	7	10	14
Age (years)	53.1±18.0 (18-84)	53.1±16.6 (18-79)	65.8±6.7 (52-80)	39.1±19.0 (17-78)	46.4±14.8 (23-62)	59.2±23.8 (23-84)	38.4±17.4 (21-77)
Gender (M:F%)	42:58	30:70	44:56	78:22	86:14	30:70	29:71
BMI (kg/m^2)	27.24±6.35 (16.65-49.20)	27.26±6.03 (16.65-37.80)	26.51±5.24 (19.00-38.02)	26.01±3.80 (21.3-33.6)	39.87±6.80 (30.0-49.2)	24.70±3.00 (20.1-28.7)	24.32±3.96 (18-31.7)
FIVC (L)	2.49±1.11 (0.40-5.42)	2.38±0.74 (1.17-3.78)	2.22±0.81 (0.71-3.59)	2.00±1.84 (0.40-5.42)	3.49±1.18 (1.41-4.74)	2.23±0.93 (0.87-3.85)	3.19±1.25 (1.02-5.25)
PIFR (l/min)	187.3±93.6 (28-456)	205.7±85.4 (59-415)	155.5±66.0 (55-275)	138.1±105.9 (28-323)	233.4±98.0 (104-389)	147.0±73.3 (35-292)	245.8±114.9 (59-456)
FEV_1 (L)	2.17±1.12 (0.24-5.07)	1.82±0.92 (0.82-4.59)	1.75±0.94 (0.24-3.80)	2.65±1.65 (0.33-5.07)	2.98±0.84 (1.58-3.97)	2.07±1.03 (0.84-3.97)	2.93±0.95 (1.02-5.07)
FVC (L)	2.88±1.19 (0.38-5.66)	2.51±0.96 (1.23-5.40)	2.58±0.98 (0.38-4.30)	3.09±1.89 (0.38-5.66)	3.71±1.05 (1.89-4.96)	2.74±1.15 (1.08-4.96)	3.65±1.11 (1.37-5.66)
FEV_1/ FVC	0.74±0.15 (0.35-0.99)	0.71±0.16 (0.44-0.94)	0.65±0.18 (0.35-0.89)	0.87±0.10 (0.71-0.99)	0.82±0.06 (0.71-0.89)	0.74±0.11 (0.50-0.87)	0.80±0.08 (0.70-0.92)

BMI – Body Mass Index, FEV_1 – Forced Expiratory Volume in 1 second, FVC – Forced Vital Capacity, PEFR – Peak Expiratory Flow Rate, FIVC – Forced Inspiratory Vital Capacity, PIFR – Peak Inspiratory Flow Rate, NMD – Neuromuscular disease, ORC – Other respiratory condition, NRC – Non-respiratory condition

Mean absolute deviation (MAD) amplitude had the strongest correlation with measured Diskus^{TM} PIFR in this varied cohort, as shown in Table 4.10.

Table 4.10: Pearson's correlation coefficients for comparisons of measured Diskus^{TM} PIFR with various amplitude parameters.

	Median amplitude (MA)	**MAD amplitude**	**RMS amplitude**	**P_{ave} in 300-600 Hz band**
Correlation coefficient, r	0.836	0.884	0.869	0.808

Based on the correlation results, PIFRc was calculated using equations derived from our previous dataset of 15 healthy volunteers in the method development study above using MAD amplitude:

$$PIFRc = \frac{194.7 * A_{MAD} + 0.1716}{A_{MAD} + 0.02621} \quad (4.1)$$

Figure 4.22 shows a scatterplot of acoustically-determined PIFR (test method) versus spirometrically-determined PIFR (reference method). Difference and relative difference plots are shown in Figure 4.23. Limits for absolute difference (+/- 1.96SD) were -11.9 to 19.4. There was a high degree of correlation between the values, with an R^2 of 0.884. There was a statistically significant mean bias of 3.78 and mean relative bias of 6.6% from the reference method.

$$PIFRc\ (l/min) = 1.01 * PIFRm\ (l/min) + 3.18 \quad (4.2)$$

The results were partitioned by PIFR values of 45, 90 and 120 l/min. There was a mean bias of 3.4 between 0-45 l/min and 3.8 between 45-90 l/min. The bias above 90 l/min was not significant.

Receiver operating characteristic (ROC) curves for various thresholds of measured PIFR are shown in Figure 4.24. AUCs are close to 1 for classification of PIFR as >= 30, 45, 60 and 90 l/min. We were able to correctly classify 95% of inhalations > 30 l/min, 91% > 45 l/min, 93% > 60 l/min and 92% > 90 l/min. Both sensitivity and specificity were greater than 90% for any threshold of measured PIFR (Table 4.11).

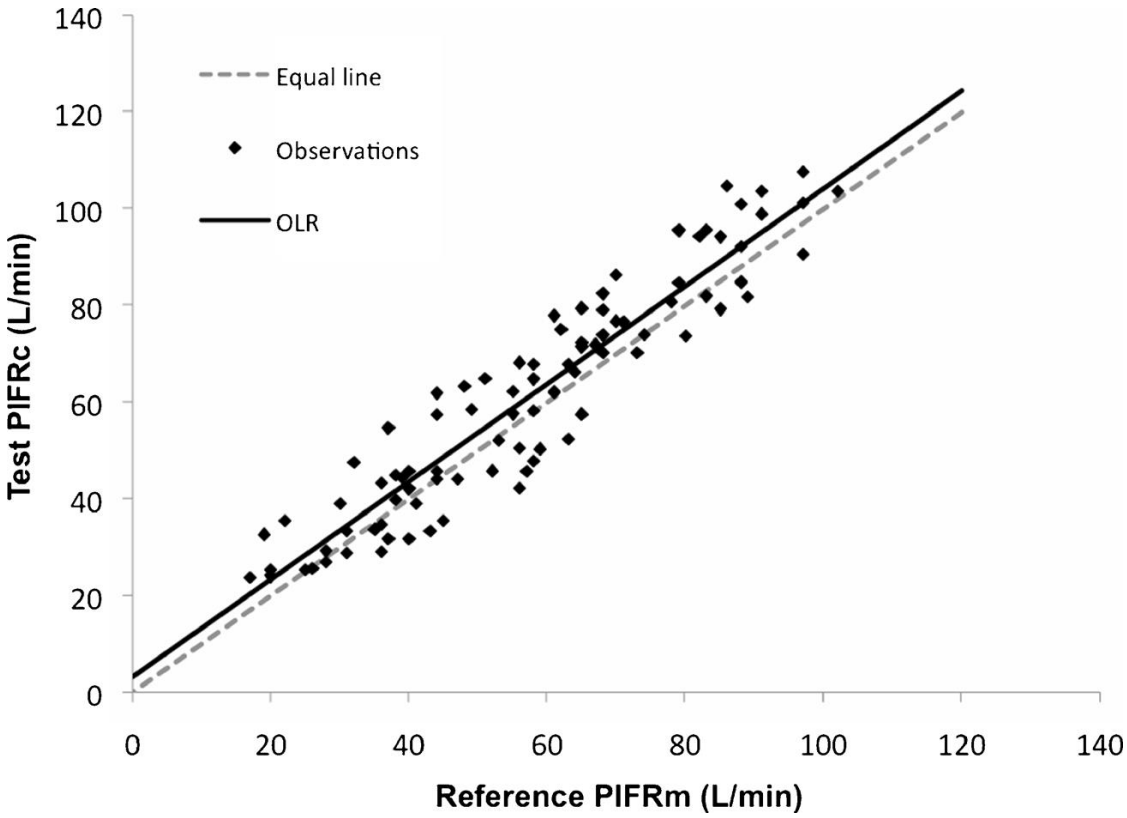

Figure 4.22: Scatter plot of test (acoustically-determined) PIFRc versus reference (spirometrically-determined) PIFRm. The equal line represents no difference between methods (y =x). The ordinary least squares regression line is also shown (R2=0.884, Test PIFR = 1.01*Reference PIFR + 3.18, Mean bias = 3.78, Mean relative bias = 6.6%).

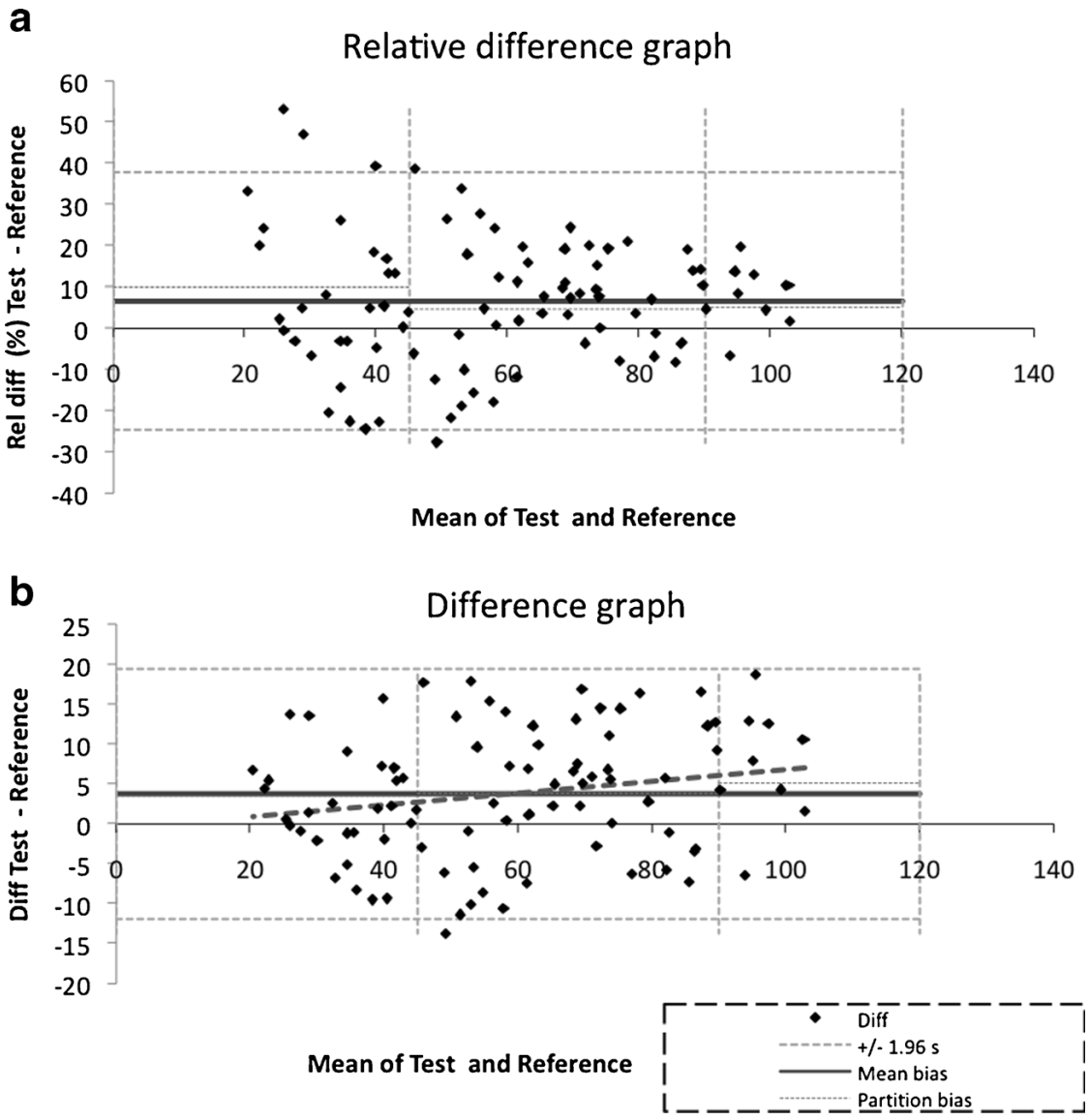

Figure 4.23: Difference (a) and relative difference (b) plots for test (acoustically-determined) PIFRc versus reference (spirometrically-determined) PIFRm.

Table 4.11: Table showing threshold values of acoustic method for which most inhalations are correctly classified, with corresponding sensitivity and specificity. Reference method represents spirometric values and test method represents acoustic method.

Reference Method (l/min)	Test Method (l/min)	Sensitivity	Specificity	Correctly Classified
≥30	≥33.55	95.12%	90.00%	94.57%
≥45	≥47.91	91.67%	90.62%	91.30%
≥60	≥66.27	90.48%	96.00%	93.48%
≥90	≥90.57	100.00%	91.86%	92.39%

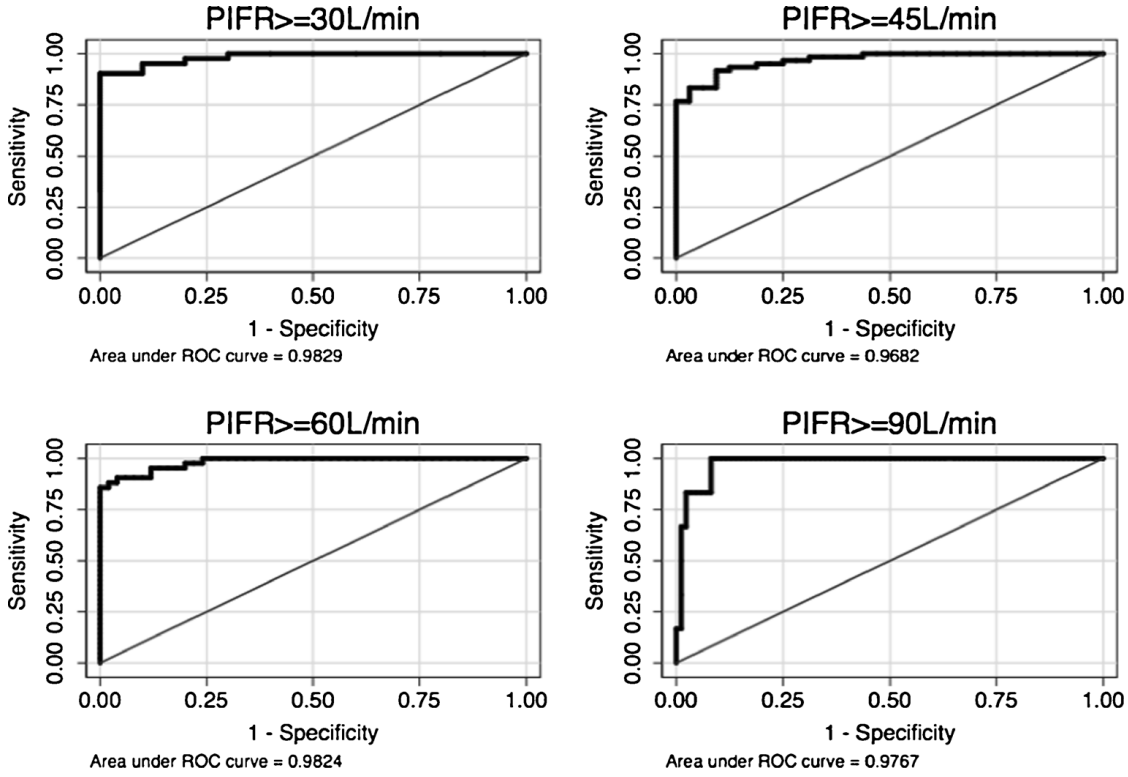

Figure 4.24: Receiver operating characteristic (ROC) curves for acoustically-determined PIFR versus thresholds of measured PIFRm of 30, 45, 60 and 90 l/min. The equal line represents an Area Under the Curve (AUC) of 0.5.

4.3.2. Discussion

In this study we extended our prior observations, which showed that analysis of the acoustics of inhalation from a Diskus^{TM} Dry Powder Inhaler could be used to calculate PIFR. Using a large sample of patients with widely varying PIFR rates, there was a very strong relationship between measured PIFR and calculated PIFR.

The results reinforced earlier findings that acoustic parameters of inhalation are both sensitive and specific for classifying inhalations according to PIFR, being able to correctly classify upwards of 89% of all inhalations according to preset thresholds of spirometrically-determined PIFR. For these analyses the sensitivities and specificities were greater than 90%. Furthermore, we have shown that the relationship between flow rate and sound amplitude is independent of disease state and is therefore applicable to a large subset of the population.

There are many ways to signal average the inhalation sound; previously, we measured the average power in the frequency band 300-600 Hz, root mean square of amplitude and mean absolute deviation of the acoustic amplitude and found that the first had the best correlation with PIFR. In this study, we found that MAD amplitude had the strongest correlation with PIFR. The most likely explanation for this is that MAD amplitude is more robust to inter-individual changes and mean power may shift in different frequency bands depending on upper and lower airway anatomy. This is in accordance with previous studies, which showed that the optimum frequency band to calculate average power is different in healthy subjects compared to asthmatics.(155)

Furthermore, we confirmed our prior findings that patients with Neuromuscular Disease and COPD generated lower PIFRs compared to asthmatics, obese patients and those with non-respiratory illnesses. This has important implications in that different sub-populations may be able to use the Diskus^{TM} inhaler with different efficacies. Even though their PIFR may be close to their

personal best, they may still not be able to generate sufficient turbulent energy to benefit from the DPI.

4.4. Correlation of inhalation acoustics from a Diskus™ Dry Powder Inhaler with in vitro drug delivery

4.4.1. Results

There was a high correlation between calculated flow rate (PIFRc) and the flow rate at which the Next Generation Impactor was operated (PIFR); overall imprecision was less than 10% at all three flow rates (Figure 4.25). Imprecision of acoustically-determined duration was approximately 3% (Figure 4.26). When regressions through the origin were performed for our data, plots of studentized residuals versus the independent variables highlighted non-horizontal linear trends indicating that a nonzero intercept should be suspected. Hence, all our regression models below included a nonzero intercept, since it is statistically significant.

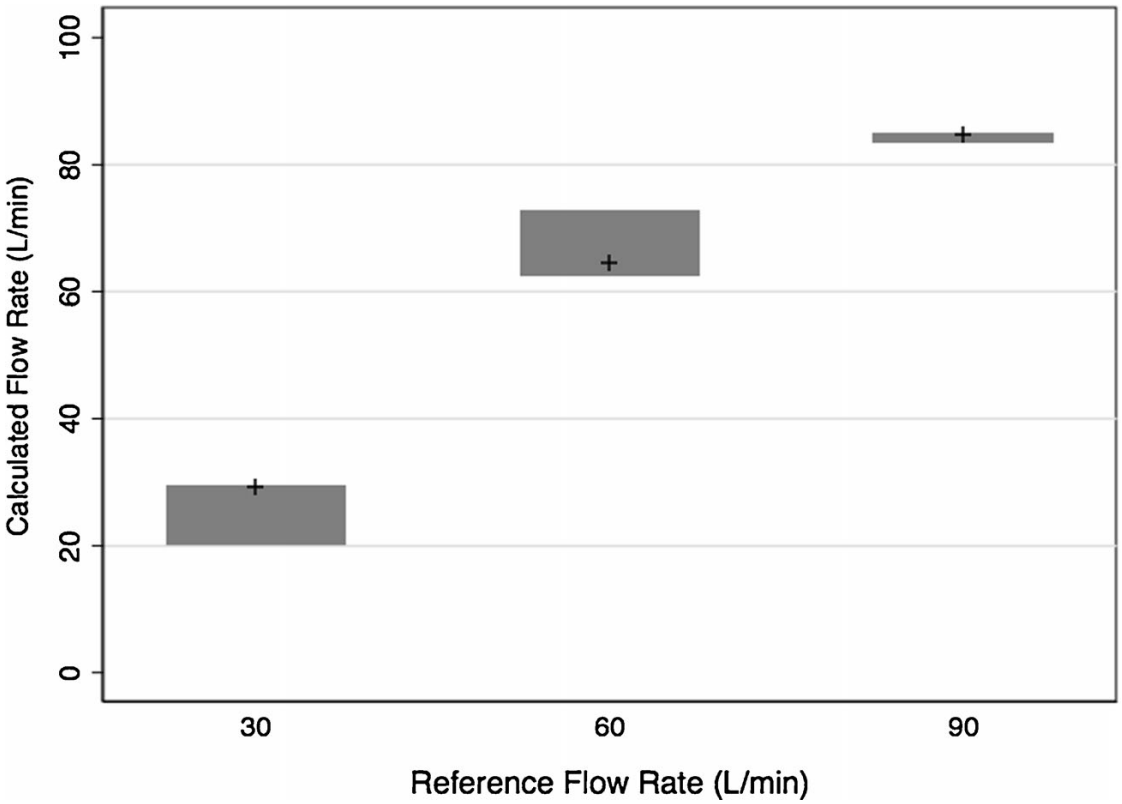

Figure 4.25: Boxplot of calculated flow rate at each preset flow rate for the NGI impactor.

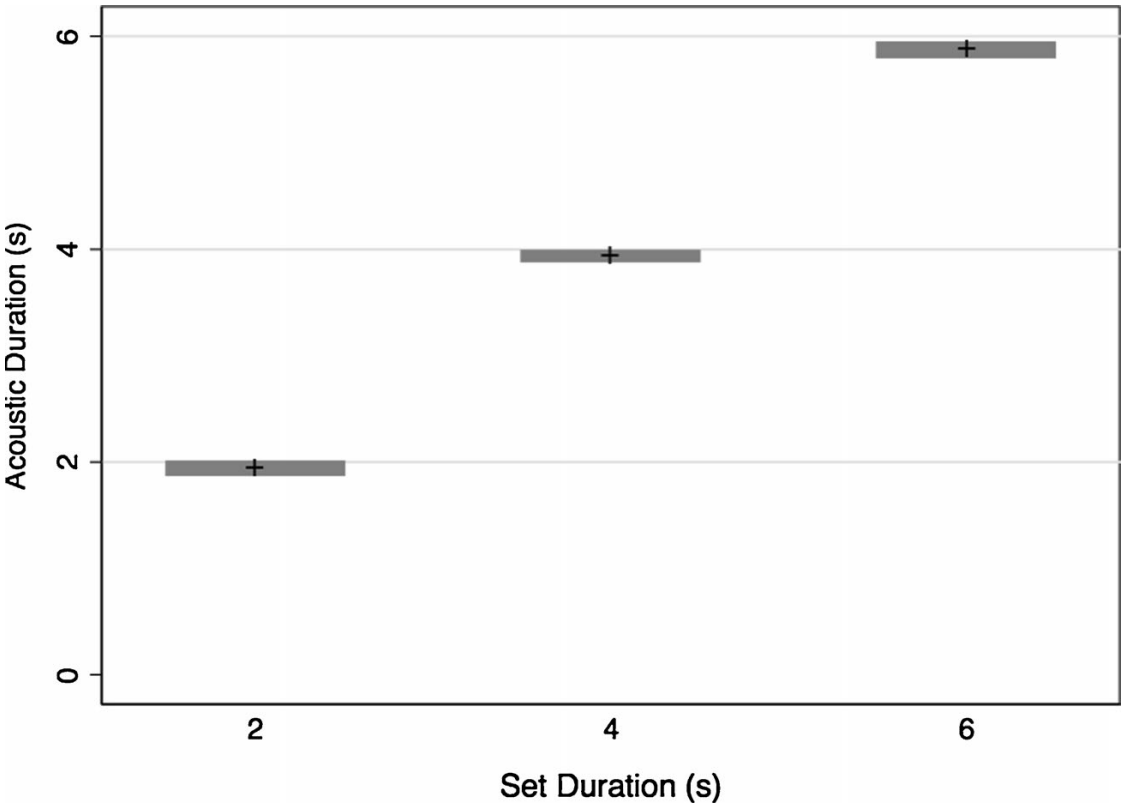

Figure 4.26: Boxplot of acoustic duration categorized by preset flow controller duration for the NGI impactor.

Fine Particle Fraction (FPF) was directly proportional to inhalation flow rate and duration of inhalation for the salmeterol/ fluticasone preparation but FPF was proportional to only PIFR for the salbutamol Diskus™ . The relationships between FPF, TED, UAD, PIFRc and duration of inhalation for salmeterol, fluticasone and salbutamol are given by the following equations:

$$\boldsymbol{Salmeterol\ FPF}\ (\%) =$$
$$0.176 * PIFRc\ (l/min) + 0.627 * Duration\ (s) + 5.915 \qquad (4.3)$$
$$Adjusted\ R^2 = 0.951$$
$$PIFRc\ (p = 0.000, \eta^2 = 0.901), Duration\ (p = 0.029, \eta^2 = 0.050)$$

$$\textbf{\textit{Salmeterol TED}}\ (\%) =$$
$$0.274 * PIFRc\ (l/min) + 72.456 \quad (4.4)$$
$$Adjusted\ R^2 = 0.858$$
$$PIFRc\ (p = 0.000, \eta^2 = 0.876), Duration\ (p = 0.139 : excluded)$$

$$\textbf{\textit{Salmeterol UAD}}\ (\%) =$$
$$0.104 * PIFRc\ (l/min) + 64.044 \quad (4.5)$$
$$Adjusted\ R^2 = 0.743$$
$$PIFRc\ (p = 0.003, \eta^2 = 0.775), Duration\ (p = 0.486 : excluded)$$

$$\textbf{\textit{Fluticasone FPF}}\ (\%) =$$
$$0.178 * PIFRc\ (l/min) + 0.640 * Duration\ (s) + 5.538 \quad (4.6)$$
$$Adjusted\ R^2 = 0.951$$
$$PIFRc\ (p = 0.000, \eta^2 = 0.901), Duration\ (p = 0.029, \eta^2 = 0.050)$$

$$\textbf{\textit{Fluticasone TED}}\ (\%) =$$
$$0.261 * PIFRc\ (l/min) + 68.581 \quad (4.7)$$
$$Adjusted\ R^2 = 0.891$$
$$PIFRc\ (p = 0.000, \eta^2 = 0.904), Duration\ (p = 0.151 : excluded)$$

$$\textbf{\textit{Fluticasone UAD}}\ (\%) =$$
$$0.046 * PIFRc\ (l/min) + 63.450 \quad (4.8)$$
$$Adjusted\ R^2 = 0.395$$
$$PIFRc\ (p = 0.041, \eta^2 = 0.470), Duration\ (p = 0.915 : excluded)$$

$$\textbf{\textit{Salbutamol FPF}}\ (\%) =$$
$$0.180 * PIFRc\ (l/min) + 29.733 \quad (4.9)$$
$$Adjusted\ R^2 = 0.7104$$
$$PIFRc\ (p = 0.001, \eta^2 = 0.747), Duration\ (p = 0.147: excluded)$$

$$\textbf{\textit{Salbutamol TED}}\ (\%) =$$
$$0.277 * PIFRc\ (l/min) + 79.524 \quad (4.10)$$
$$Adjusted\ R^2 = 0.725$$
$$PIFRc\ (p = 0.002, \eta^2 = 0.760), Duration\ (p = 0.124 : excluded\ )$$

$$\boldsymbol{Salbutamol\ UAD}\ (\%) =$$
$$0.0970 * PIFRc\ (l/min) + 49.790 \qquad (4.11)$$
$$Adjusted\ R^2 = 0.445$$
$$PIFRc\ (p = 0.031, \eta^2 = 0.515), Duration\ (p = 0.253 : excluded)$$

While both calculated flow rate and acoustic duration are statistically significant in the regression models for FPF from the salmeterol/ fluticasone inhaler, inhalation duration has a minimal effect compared to PIFR as estimated by the η^2. Duration was not a significant variable in the FPF model for salbutamol and all of the models for TED and UAD (see equations above for p values). The trends for TED were similar to those seen with FPF (Figures 4.27 and 4.28).

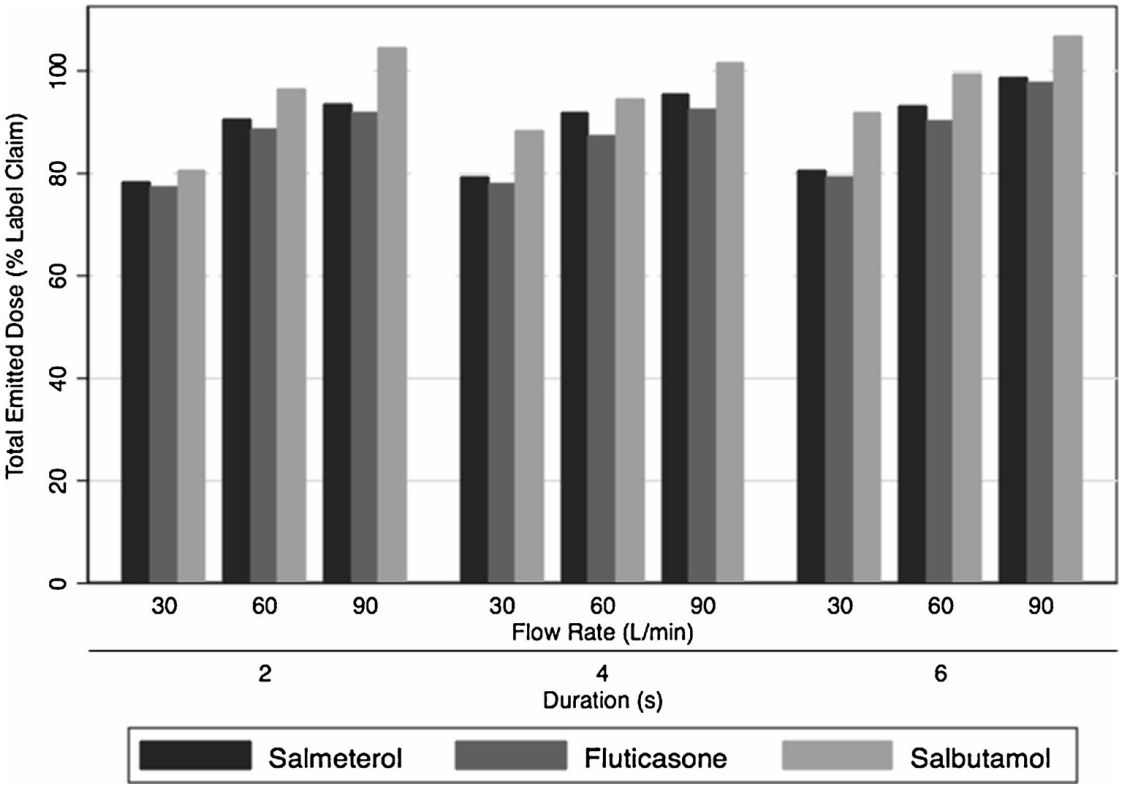

Figure 4.27: Bar graph of average total emitted dose (n=2, %RSD < 20%) as a % of label claim versus calculated flow rate for salmeterol, fluticasone and salbutamol for (a) 2 s inhalation, (b) 4 s inhalation and (c) 6 s inhalation.

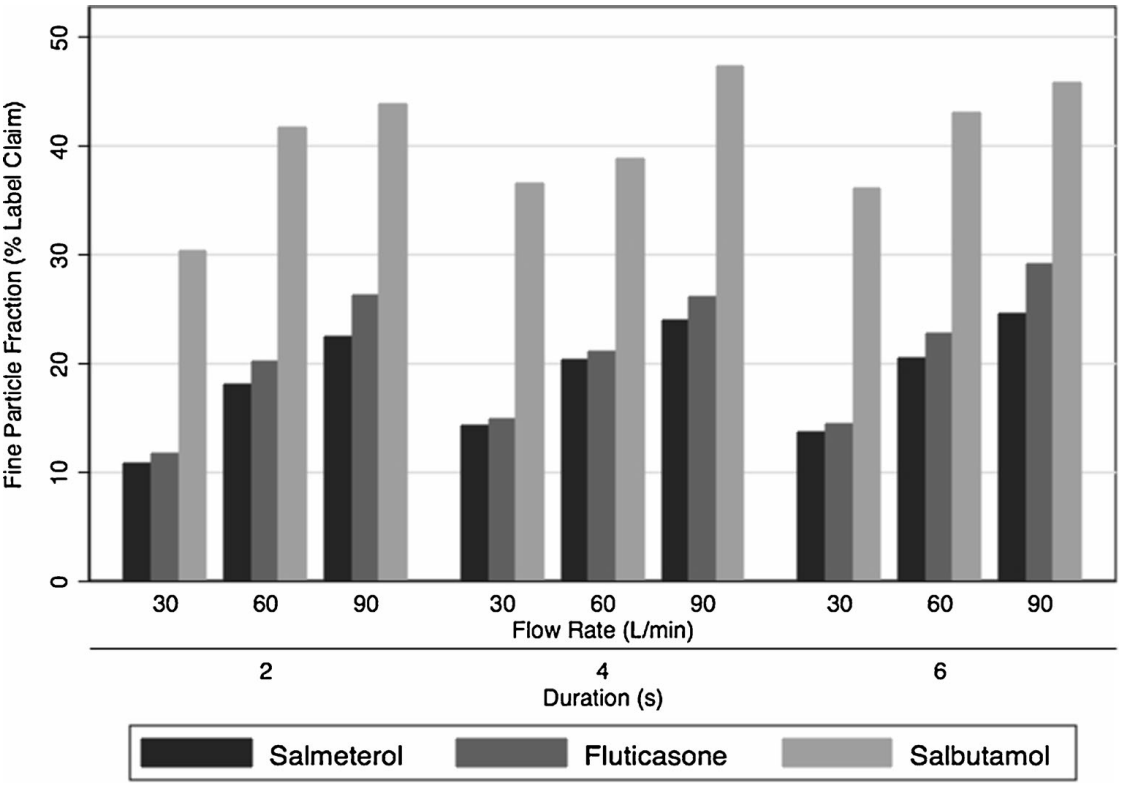

Figure 4.28: Bar graph of average fine particle fraction (n=2, %RSD < 20%) as a % of label claim versus calculated flow rate for salmeterol, fluticasone and salbutamol for (a) 2 s inhalation, (b) 4 s inhalation and (c) 6 s inhalation.

A significant proportion of active drug is of a diameter greater than 5 microns and hence, likely to be deposited in the upper airways and throat (Figure 4.29). PIFRc is only moderately correlated with UAD, with an adjusted R^2 of 0.708 for salmeterol, 0.295 for fluticasone and 0.527 for salbutamol. Inhalation duration had no effect on UAD.

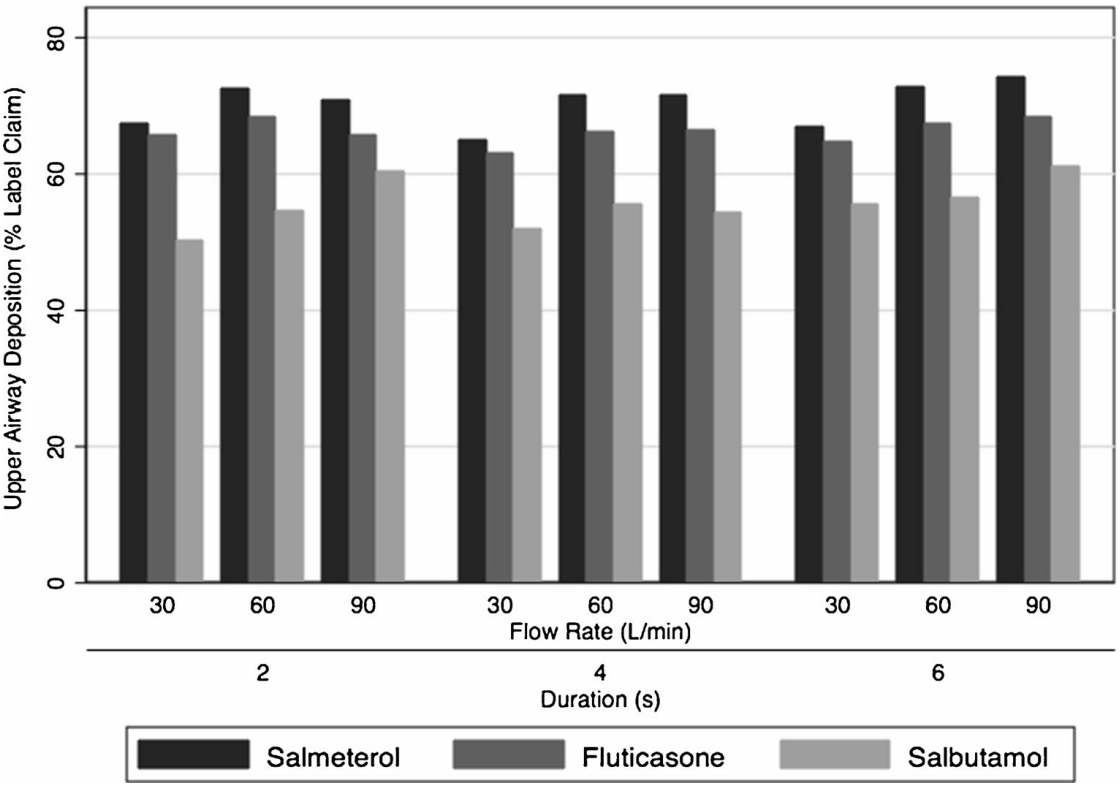

Figure 4.29: Bar graph of average upper airway deposition (n=2, %RSD < 20%) as a % of label claim versus calculated flow rate for salmeterol, fluticasone and salbutamol for (a) 2 s inhalation, (b) 4 s inhalation and (c) 6 s inhalation.

Tables 4.12, 4.13 and 4.14 present the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) for salmeterol, fluticasone and salbutamol according to flow rate and duration of inhalation. There is a clear trend to a lower MMAD at higher flow rates for both Diskus^{TM} formulations. However, the GSD or spread of particle diameters increases as flow rate increases from 30 l/min to 90 l/min. The MMAD is also consistently lower for the salbutamol formulation under all study conditions.

Table 4.12: Next Generation Impactor salmeterol deposition by flow rate and duration of inhalation.

Salmeterol									
Flow Rate (l/min)	**30**			**60**			**90**		
Duration (s)	**2**	**4**	**6**	**2**	**4**	**6**	**2**	**4**	**6**
Throat (µg)	6.66	5.37	5.81	5.96	6.43	4.99	5.36	5.40	4.51
PS[a] (µg)	24.60	24.05	24.73	27.81	27.05	28.57	27.36	27.36	29.53
S1 (µg)	1.03	1.10	1.03	2.01	2.03	2.40	3.87	4.25	4.20
S2 (µg)	1.72	2.42	2.28	3.37	3.35	3.82	3.85	4.07	4.55
S3 (µg)	2.50	3.15	2.88	3.30	3.42	3.75	3.36	3.61	3.50
S4 (µg)	2.05	2.76	2.69	2.32	2.68	2.50	2.37	2.48	2.47
S5 (µg)	BLOQ	0.74	0.65	BLOQ	0.87	BLOQ	BLOQ	BLOQ	BLOQ
S6 (µg)	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
S7 (µg)	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
MOC[b] (µg)	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
TED[c] (µg)	39.07	39.60	40.23	45.21	45.82	46.60	46.62	47.65	49.32
FPD[d] (µg)	5.39	7.13	6.63	9.26	10.15	10.48	11.86	12.67	13.00
MMAD[e] (µm)	5.18	5.08	4.95	4.26	4.03	4.32	3.83	3.86	3.91
GSD[f]	1.77	1.80	1.88	1.81	1.89	1.87	2.23	2.26	2.12

[a] PS – Pre-separator, [b] MOC – Micro-orifice Collector, [c] TED – Total Emitted Dose, [d] FPD – Fine Particle Dose, [e] MMAD – Mass Median Aerodynamic Diameter, [f] GSD – Geometric Standard Deviation, BLOQ – Below Limit of Quantification (0.63 micrograms)

Table 4.13: Next Generation Impactor fluticasone deposition by flow rate and duration of inhalation.

Fluticasone									
Flow Rate (l/min)	**30**			**60**			**90**		
Duration (s)	**2**	**4**	**6**	**2**	**4**	**6**	**2**	**4**	**6**
Throat (µg)	32.72	25.17	28.54	29.77	32.11	23.98	26.64	27.92	23.29
PS[a] (µg)	119.13	116.82	118.02	130.41	123.50	131.70	128.31	128.04	137.15
S1 (µg)	5.13	5.29	5.04	9.74	9.45	11.10	18.12	19.50	19.65
S2 (µg)	8.58	12.05	11.58	16.69	13.42	19.45	20.25	20.65	24.74
S3 (µg)	13.05	16.73	15.23	17.92	17.97	21.16	19.60	18.66	20.88
S4 (µg)	11.22	14.00	15.26	13.77	13.08	13.86	13.91	13.58	14.94
S5 (µg)	3.38	4.52	3.66	2.98	8.35	3.82	2.84	2.84	2.96
S6 (µg)	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
S7 (µg)	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
MOC[b] (µg)	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
TED[c] (µg)	193.22	194.59	197.54	221.28	217.87	225.07	229.68	231.19	243.62
FPD[d] (µg)	28.83	36.82	35.48	50.35	52.53	56.93	65.42	65.11	72.64
MMAD[e] (µm)	4.98	4.98	4.84	4.03	3.63	4.10	3.47	3.65	3.63
GSD[f]	1.79	1.78	1.87	1.82	1.86	1.77	2.22	2.23	2.08

[a] PS – Pre-separator, [b] MOC – Micro-orifice Collector, [c] TED – Total Emitted Dose, [d] FPD – Fine Particle Dose, [e] MMAD – Mass Median Aerodynamic Diameter, [f] GSD – Geometric Standard Deviation, BLOQ – Below Limit of Quantification (1.50 micrograms)

Table 4.14: Next Generation Impactor salbutamol deposition by flow rate and duration of inhalation.

Salbutamol									
Flow Rate (l/min)	**30**			**60**			**90**		
Duration (s)	**2**	**4**	**6**	**2**	**4**	**6**	**2**	**4**	**6**
Throat (µg)	14.31	14.77	15.38	15.13	15.18	23.15	19.52	21.88	18.61
PS[a] (µg)	60.89	62.56	67.96	77.88	79.28	67.38	87.57	73.21	90.58
S1 (µg)	15.00	15.29	17.45	15.32	15.45	18.97	17.56	17.21	16.40
S2 (µg)	13.42	15.26	14.29	11.27	11.93	17.26	13.38	13.73	13.05
S3 (µg)	19.48	20.14	22.03	19.12	16.95	21.78	14.98	16.09	16.33
S4 (µg)	21.90	24.67	23.16	25.90	24.94	29.68	18.77	22.58	21.30
S5 (µg)	10.42	11.49	11.68	14.14	12.43	13.74	15.27	18.46	16.13
S6 (µg)	4.12	3.64	3.87	5.59	3.78	2.45	6.77	6.68	6.90
S7 (µg)	BLOQ	3.66	3.76	3.59	3.81	2.14	6.85	6.30	6.60
MOC[b] (µg)	BLOQ	5.20	3.69	4.62	4.74	2.04	7.72	6.75	7.47
TED[c] (µg)	160.64	176.68	183.26	192.57	188.49	198.59	208.39	202.90	213.37
FPD[d] (µg)	60.52	72.90	72.08	83.29	77.49	85.87	87.66	94.55	91.41
MMAD[e] (µm)	4.51	4.08	4.32	2.60	2.67	3.06	1.92	1.87	1.88
GSD[f]	2.24	2.44	2.29	2.17	2.12	2.42	2.94	2.59	2.66

[a] PS – Pre-separator, [b] MOC – Micro-orifice Collector, [c] TED – Total Emitted Dose, [d] FPD – Fine Particle Dose, [e] MMAD – Mass Median Aerodynamic Diameter, [f] GSD – Geometric Standard Deviation, BLOQ – Below Limit of Quantification (2.00 micrograms)

4.4.2. Discussion

To confirm that drug delivery to the lungs is dependent on flow rate, and hence can be estimated from the acoustic sounds of inhalation, we performed *in vitro* and *in vivo* studies. *In vitro*, we showed that Fine Particle Dose was dependent on both the inhalation flow rate and the duration of inhalation for salmeterol and fluticasone; duration was not significant for salbutamol FPF. Using the acoustic parameters to determine PIFRc and the duration of inhalation, we were able to explain more than 95% of the variance in FPF for salmeterol/ fluticasone but only 70% of the variance for salbutamol. In contrast, the Upper Airway Deposition was relatively constant regardless of flow rate and duration. The implications of this is that patients with poor inhalational technique may have all the side effects of thrush and GI absorption with very few beneficial effects of the medication.

Some authors have described the Diskus^{TM} DPI as flow-independent.(91) However, on careful review of their results, FPF from the Diskus^{TM} DPI is flow-dependent, although not to the same degree as that from the Turbuhaler^{TM} DPI. There is little published data on the effect of duration or inhaled volume on drug delivery. Our data suggest that the effect of inhalation duration is minimal. However, duration is still a significant variable in our regression models for salmeterol and fluticasone FPF and it is likely that at borderline flow rates between 30-45 l/min, inspiratory duration plays a more important role in drug delivery. Further studies at inhalation durations less than or equal to 1 second are required to further evaluate any possible relationship.

A number of studies have reported that very high inhalation flow rates through the Diskus^{TM} inhaler may be detrimental to airway drug delivery, arguing that throat deposition is increased and that particles less than 1 micron in size are more likely to be exhaled immediately after inhalation.(94, 164) In contrast, our study found that even though MMAD decreases as flow rate increases, the lowest MMAD achieved for the salmeterol/fluticasone Diskus^{TM} was 3.47 mm with a GSD of 2.22, which means that a significant proportion of particles

would still be in the range of 2-5 microns to be active on the small airways. It is worth mentioning that the MMAD values for salbutamol were lower than the salmeterol/ fluticasone formulation. Hence, for this formulation, PIFRs >60 l/ min from a salbutamol Diskus™ may lead to lower pulmonary deposition due to exhalation of particles < 1 micron.

One of the limitations of cascade impactor studies is that they require multiple dose actuations in order to enhance detection of very low drug levels in the lower Stages. This increases the chances of particle re-entrainment with each subsequent inhalation and hence, the drug recovered in each stage is likely to be higher than that expected if only one actuation were performed.

4.5. Correlation of inhalation acoustics from a Diskus™ Dry Powder Inhaler with in vivo drug delivery

4.5.1. Results

Baseline demographics for the ten subjects recruited in this study are shown in Table 4.15. Figure 4.30 shows that there was a significant difference between peak salbutamol concentration (measured at 20 min) achieved when PIFR was above 60 l/min compared to when PIFR was below 60 l/min for each individual. A t-test for difference in means of groups above and below 60 l/min gave a *p* value < 0.0001 with a mean difference of 0.786 (95% CI: 0.472 – 1.100).

Table 4.15: Demographics of ten healthy volunteers recruited for pharmacokinetic study.

Demographic	All subjects
Age (years), mean (SD)	31.1 (9.6)
Sex, M:F (%)	70:30
Body mass index (kg/m^2), mean (SD)	23.1 (2.7)
Height (cm), mean (SD)	173.7 (10.0)
Weight (kg), mean (SD)	69.5 (9.8)
Race, n (%)	
White – White/ Caucasian/ European	8 (80)
Asian – Central/ South Asian	2 (20)

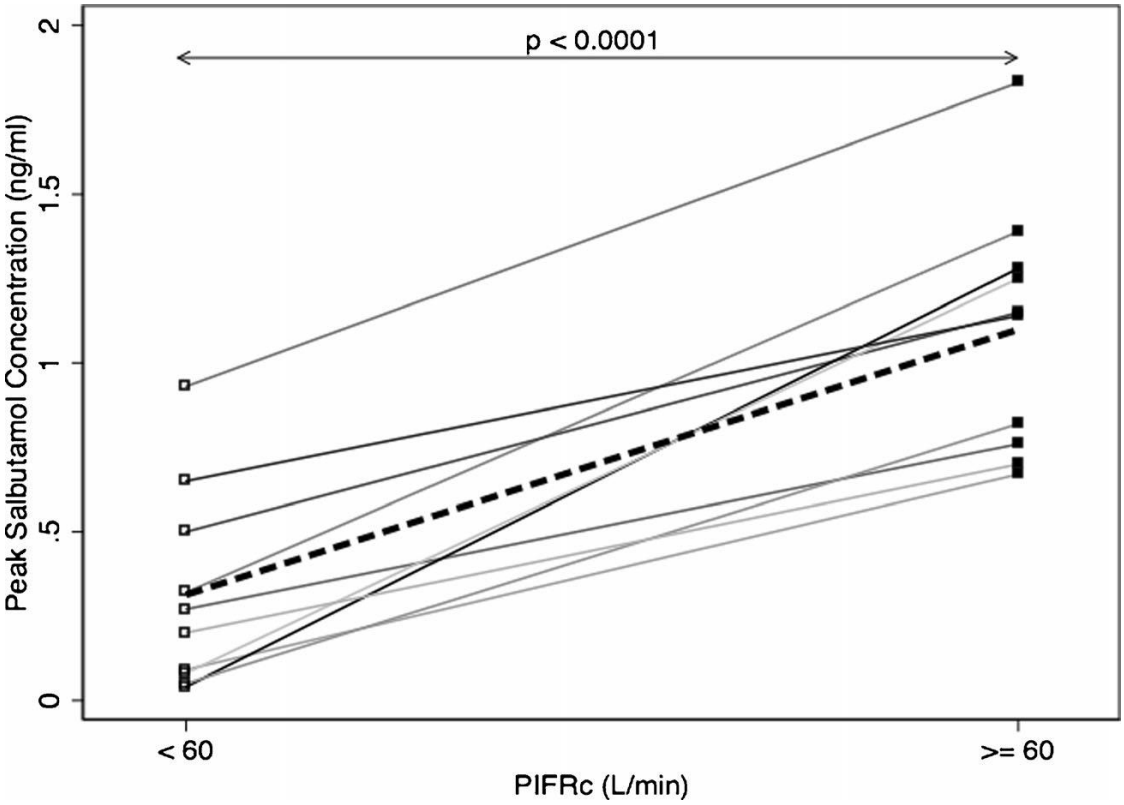

Figure 4.30: Line and dot plot of peak serum concentration of salbutamol versus flow rate category (less than or greater than 60 l/min) for ten healthy subjects. Each line represents a separate individual and points represent actual values of concentration and calculated PIFR. The dotted line represents the overall regression line for all the data points. P- value for difference in means between high flow rate and low flow rate groups is less than 0.0001.

Duration of inhalation, and by extension, inspiratory volume did not significantly contribute to the multi-level regression model. The R^2 for the clustered regression model was 0.563 ($p < 0.0001$), with standard error adjusted for 10 clusters of subjects. A large proportion of the variance in peak salbutamol concentration could not be explained by inhalation flow rate and duration.

4.5.2. Discussion

The relationship between PIFR and duration of inhalation on drug delivery, *in vivo*, was tested in ten healthy subjects. There was a significant difference in the serum concentrations of salbutamol when PIFR was low (≤ 60 l/min) compared to when the PIFR was > 60 l/min. Together these data confirm that the acoustics of inhalation from a Diskus™ DPI can be used to objectively quantify pulmonary drug delivery.

A salbutamol Diskus™ was used because salbutamol has the shortest half-life of the drugs studied in vitro in section 4.4. and it reaches relatively high concentrations in the blood after inhalation with a short time to maximum concentration. It was straightforward to measure serum plasma concentrations using a commercially available ELISA. In preliminary experiments there was an initial peak at 20 minutes that was distinct from the peak at 2-3 hours, which is likely secondary to GI absorption. The initial peak was therefore most likely related to pulmonary absorption and hence, pulmonary deposition and aerodynamic particle size. Results were concordant with the *in vitro* studies using the NGI and confirmed the relationship between PIFR and peak blood concentration.

Each subject served as his or her own control since inter-individual drug metabolism is highly variable. Each individual achieved a lower C_{max} when his or her inhalation flow rate was less than 60 l/min. Furthermore, the equations developed to estimate PIFR from acoustics were able to correctly classify all of the inhalations as either above or below 60 l/min and acoustically-determined PIFR explained more than 50% of the variance in C_{max}. The remainder of the variance is likely due to differences in drug metabolism between individuals. The study was underpowered to detect a relationship between duration of inhalation and peak concentration. The existence of such a relationship is however, questionable since the results of prior *in vitro* studies were inconclusive (even though the results for salmeterol and fluticasone were statistically significant, the magnitude of the effect is minimal).

One limitation of this pharmacokinetic study was the use of salbutamol without giving charcoal to the subjects to minimize GI absorption. A consensus statement from the British Association for Lung Research recommends the use of an inhaled drug like fluticasone, which has less than 1% oral bioavailability, in pharmacokinetic studies or another drug in combination with activated charcoal.(165) However, we based our method on a previous study, which showed that mouth-rinsing effectively eliminates GI absorption.(37) Our data from three volunteers also shows that the peak due to GI absorption happens much later than when we collected our blood samples. While it would have been ideal to use an HPLC or LC-MS/MS assay for detection of salbutamol, this technology was not available at the time sample analysis was performed. Nonetheless, our method validation of the ELISA showed that is had an acceptable imprecision and good recovery.

CHAPTER 5: Development and validation of an acoustic method to detect and quantify the effect of exhalation into a Dry Powder Inhaler

5.1. Results

5.1.1. Dosage Uniformity Analysis

The dosage uniformity analysis on the Diskus™ DPI demonstrated that the dose delivered from the Diskus™ was uniform and repeatable. Nine of 10 test results fell between 75% - 125% and 1 of 10 test results was between 65% -135% of the delivered dose label claim, which was in accordance with US Pharmacopoeia standards. Results for this testing can be found in Table 5.1.

Table 5.1: Dosage uniformity analysis of salmeterol/ fluticasone Diskus™ performed for further comparisons.

	Salmeterol Delivered Dose (mcg)	Salmeterol Delivered Dose (% label claim)	Fluticasone Delivered Dose (mcg)	Fluticasone Delivered Dose (% label claim)
DUSA 1	37.14	74.28	200.73	80.29
DUSA 2	43.79	87.58	225.66	90.27
DUSA 3	40.86	81.73	207.45	82.98
DUSA 4	47.55	95.11	231.39	92.56
DUSA 5	47.43	94.87	238.31	95.32
DUSA 6	47.07	94.14	239.23	95.69
DUSA 7	46.56	93.13	238.56	95.42
DUSA 8	46.46	92.93	218.92	87.57
DUSA 9	48.37	96.75	245.06	98.02
DUSA 10	47.95	95.90	242.10	96.84
Average	**45.32**	90.64	**228.74**	91.50

5.1.2. Impact of exhalation on delivered dose

The impact of four distinct exhalation factors (exhalation flow rate, distance to inhaler mouthpiece, exhalation duration and relative air humidity level) on drug delivery was investigated. It was found from multivariate regression analysis that all four factors had a statistically significant effect on both salmeterol and fluticasone drug delivery ($p<0.05$, significance level $\alpha=0.05$). From the multivariate regression model, the adjusted R-squared values were 62.7% for salmeterol and 63.4% for fluticasone. The multivariate regression equations derived can be found below:

$$\boldsymbol{Salmeterol\ Delivered\ Dose}\ (ug) = -0.153 * Exhalation\ flow\ rate\ (L/min) + 2.146 * Distance\ from\ inhaler\ (cm) - 1.840 * Duration\ of\ exhalation\ (s) - 10.372 * Air\ condition\ (dry = 0/\ humid = 1) + 43.310 \quad (5.1)$$

$$\boldsymbol{Fluticasone\ Delivered\ Dose}\ (ug) = -0.728 * Exhalation\ flow\ rate\ (L/min) + 10.715 * Distance\ from\ inhaler\ (cm) - 9.008 * Duration\ of\ exhalation\ (s) - 50.634 * Air\ condition\ (dry = 0/\ humid = 1) + 214.997 \quad (5.2)$$

Figure 5.1 details the total percentage of salmeterol and fluticasone delivered as a percentage of the Diskus™ inhaler manufacturer's claims. Scatterplot matrices are also shown to illustrate the differences due by relative air humidity level.

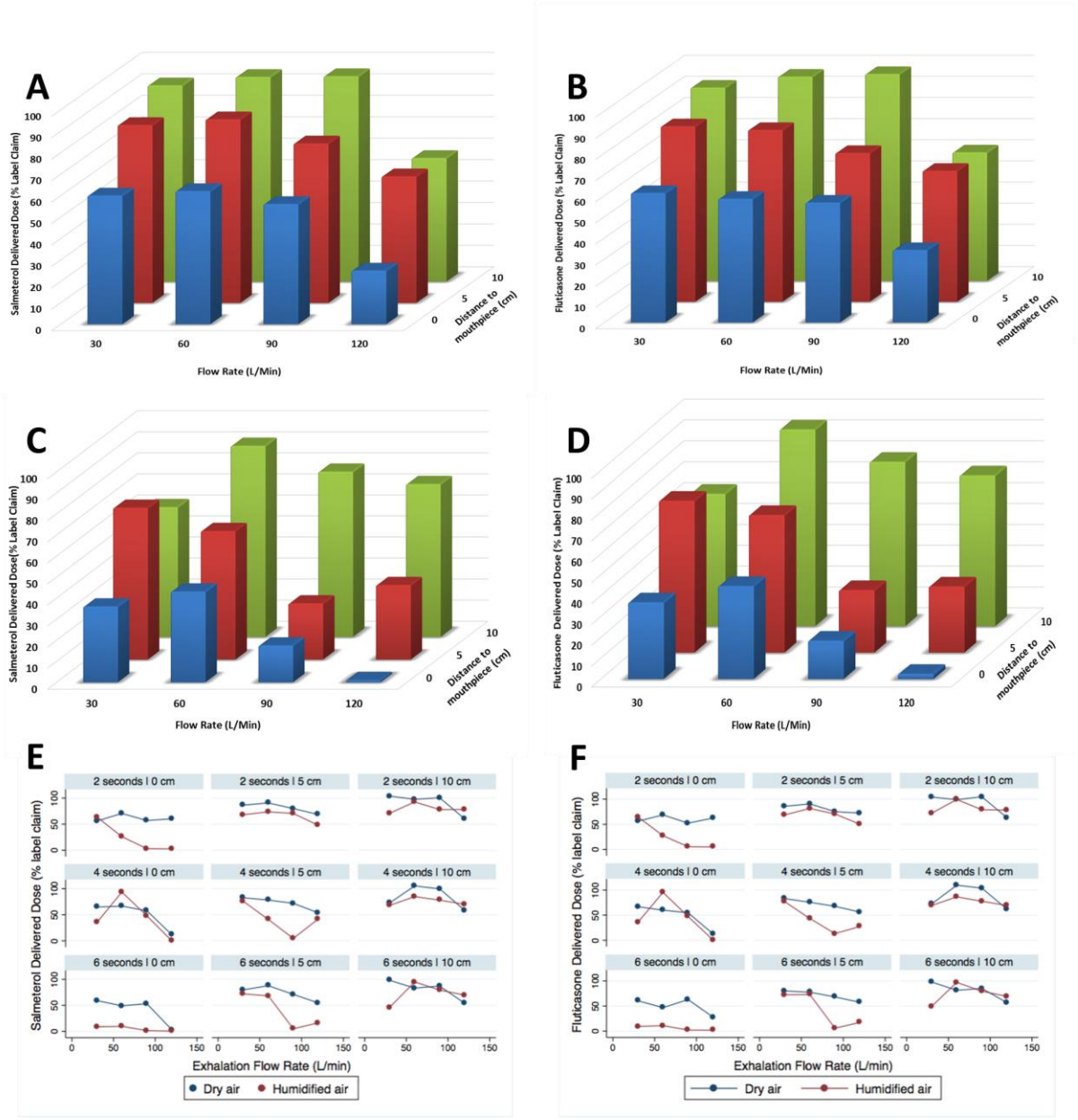

Figure 5.1: Effect of exhalations on delivered dose as percentage of label claim: (A) salmeterol delivered dose after exhalation with dry air, (B) fluticasone delivered dose after exhalation with dry air, (C) salmeterol delivered dose after exhalation with humid air, (D) fluticasone delivered dose after exhalation with humid air, (E) interaction plot detailing differences between salmeterol delivered dose for different factors and (F) scatterplot matrices detailing differences between fluticasone delivered dose for different factors. Experiments were performed in triplicate (n=3) at each study condition.

Exhalations were found to have an overall negative effect on drug delivery. At a distance of 0 cm from the inhaler mouthpiece, less than 50% of drug available was delivered on average for all flow rates using humid air (relative air humidity = 80%). In a worst-case scenario, an average of 2.44% of drug was delivered at an expiratory flow rate of 120 l/Min, at a distance of 0 cm from the inhaler mouthpiece (Figure 5.1 C & D). Delivered dose was more consistent when dry air was used and more variable and unpredictable when humid air was used. Less drug was delivered on average when humid air was used in comparison to dry air (Figure 5.1 E & F).

To investigate the effects of each of the four factors on drug delivery, measures of effect size (η^2 and partial η^2) were obtained from the multivariate regression model for each independent variable. Results established that distance from the inhaler mouthpiece was the single most influential factor in reducing the percentage of drug delivery from a DPI. Exhalation flow rate and air humidity level were the next most influential factors with similar effect sizes. Lastly, although its overall effect was significant, exhalation duration was the least influential factor in determining drug delivery for the multivariate regression model. Results for this analysis are presented in Tables 5.2 and 5.3 respectively.

Table 5.2: Effect size for each of the four factors on drug delivery for salmeterol.

Variable	P > |t|	η^2	% Change in η^2	Partial η^2
Exhalation flow rate	0.000	0.122	18.890	0.258
Distance	0.000	0.358	55.255	0.504
Duration	0.006	0.042	6.498	0.107
Air Humidity	0.000	0.125	19.356	0.262

Table 5.3: Effect size for each of the four factors on drug delivery for fluticasone.

Variable	P > |t|	η^2	% Change in η^2	Partial η^2
Exhalation flow rate	0.000	0.116	17.723	0.251
Distance	0.000	0.372	56.819	0.519
Duration	0.006	0.042	6.426	0.109
Air Humidity	0.000	0.125	19.032	0.265

5.1.3. Particle size distribution of emitted dose

An NGI cascade impactor was employed to investigate the effect of exhalations versus no exhalations on the particle distribution from a Diskus™ DPI. There were no differences in the total emitted doses but the fine particle fraction (FPF) was significantly reduced for inhaler devices subjected to an exhalation, see Figure 5.2. Detailed results for particle size distribution can be found in Table 5.4.

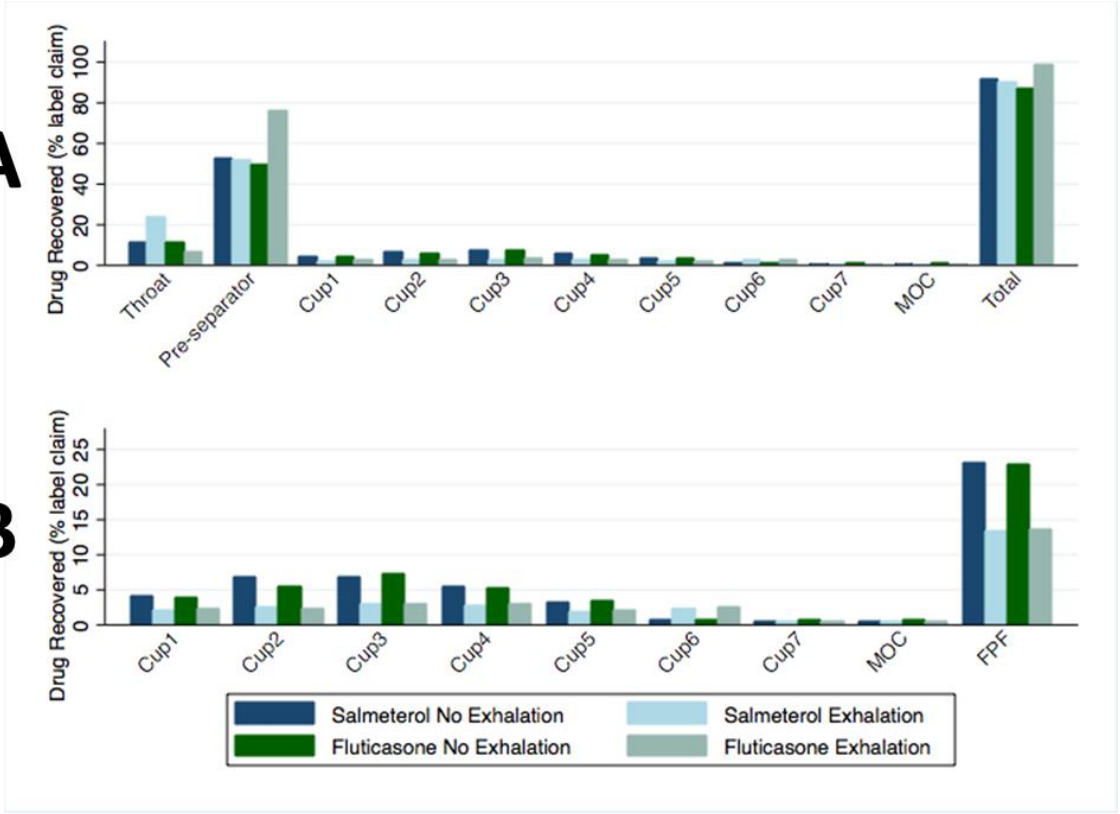

Figure 5.2: Analysis of particle size distribution of salmeterol and fluticasone from Diskus™ DPI as obtained from NGI. (A) Total drug recovered from all sections of the NGI and (B) Fine Particle Fraction (FPF) drug recovered demonstrating a reduction due to exhalations. Experiments were performed in duplicate (n=2) at each study condition.

Table 5.4: Particle size distribution from NGI for salmeterol and fluticasone with and without an exhalation.

Active ingredient	**Salmeterol**		**Fluticasone**	
Study Condition	**Standard Dose**	**Post humidified-air exhalation**	**Standard Dose**	**Post humidified-air exhalation**
Throat (µg)	5.60	11.90	27.35	16.92
PS[a] (µg)	26.40	25.68	123.50	190.17
S1 (µg)	2.03	1.03	9.45	5.63
S2 (µg)	3.35	1.19	13.42	5.63
S3 (µg)	3.42	1.43	17.97	7.45
S4 (µg)	2.68	1.37	13.08	7.20
S5 (µg)	1.52	0.93	8.35	5.03
S6 (µg)	BLOQ	1.13	1.72	6.05
S7 (µg)	BLOQ	BLOQ	1.51	BLOQ
MOC[b] (µg)	BLOQ	BLOQ	1.52	BLOQ
TED[c] (µg)	45.82	45.06	217.87	245.92
FPD[d] (µg)	11.53	6.63	57.01	33.87
UAD[e] (µg)	34.29	38.42	160.86	212.05
MMAD[f] (µm)	3.85	2.92	3.39	2.86
GSD[g]	1.97	2.40	1.95	2.39

[a] PS – Pre-separator, [b] MOC – Micro-orifice Collector, [c] TED – Total Emitted Dose, [d] FPD – Fine Particle Dose, [e] MMAD – Mass Median Aerodynamic Diameter, [f] GSD – Geometric Standard Deviation, BLOQ – Below Limit of Quantification

5.1.4. Relationship between exhalations and acoustic features

A strong correlation was observed between the exhalation flow rate and the acoustic features obtained from the exhalation audio signal. As the expiratory flow rate increased, a corresponding increase was seen in both P_{ave} in the 300-600 Hz frequency band and in the MAD amplitude. Distance between the inhaler and the artificial mouthpiece was also related to both power and amplitude of the exhalation signal. The smaller this distance, the greater the power and amplitude of the signal. Results for the correlations between acoustic features with both flow rate and distance are illustrated in Figure 5.3.

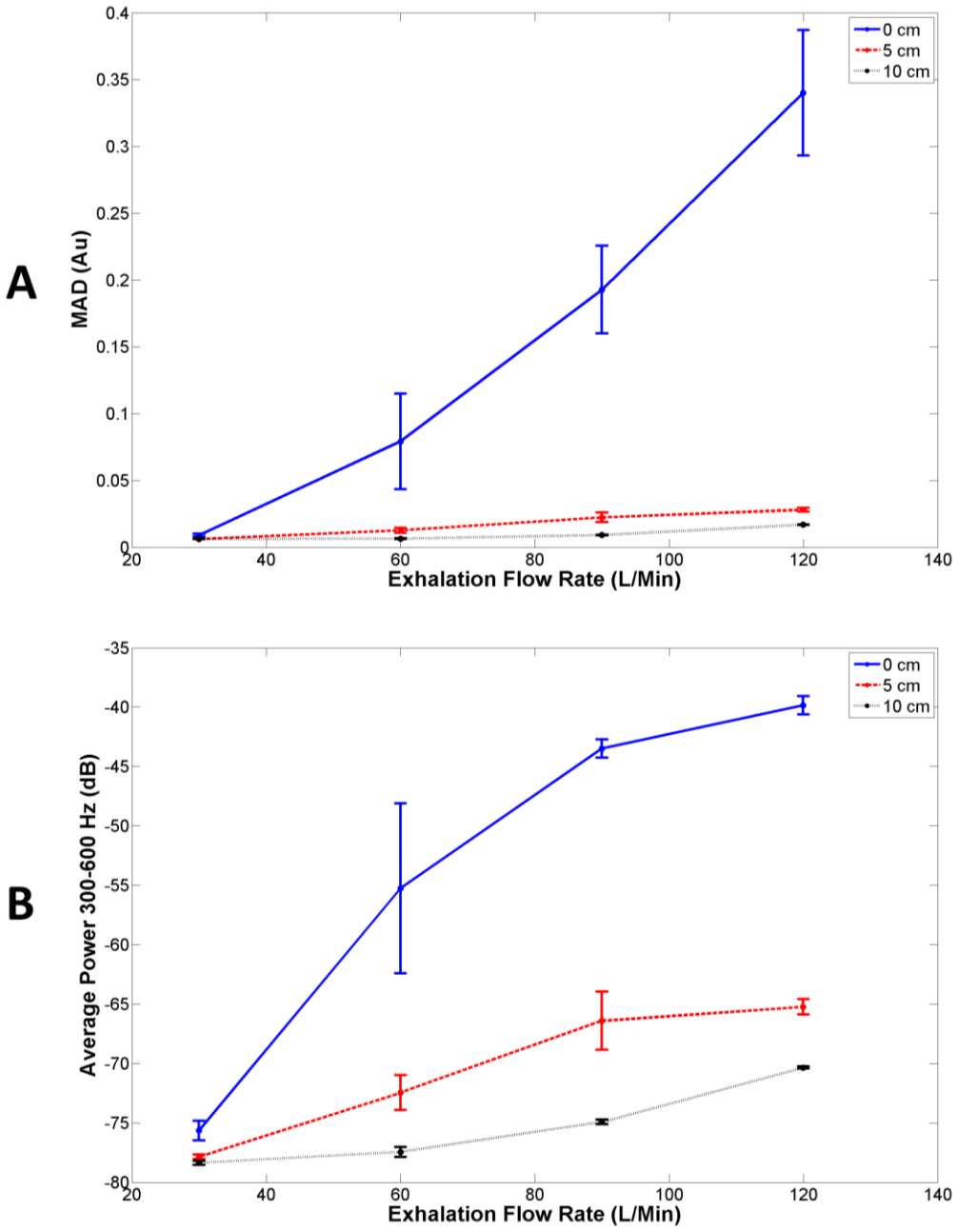

Figure 5.3: Correlations between flow and distance from inhaler mouthpiece with acoustic features for humid air *in vitro*. (A) Mean Absolute Deviation (MAD) of the exhalation signal plotted versus exhalation flow rate and distance form inhaler mouthpiece. (B) Average power in the 300-600 Hz frequency band of the exhalation signal plotted versus exhalation flow rate and distance form inhaler mouthpiece.

5.1.5. Acoustic method of automatically detecting exhalations

Cohen's kappa statistic (K) was calculated to measure the level of agreement between the two respiratory clinicians who manually classified each audio file in the validation dataset. K was found to be 1, indicating perfect agreement between the two human raters. Using the algorithm described to automatically detect exhalations, an evaluation test was performed on the 120 audio files from 22 patients, which made up the validation dataset. The overall detection rate (accuracy) on the 22 patients in the validation dataset was found to be 89.1% compared to the gold standard method of classification. Sensitivity (detecting exhalations as exhalations) was found to be 82.2%, and specificity (detecting noise as noise) was found to be 91.6% compared to the gold standard method. These results demonstrated that the algorithm developed may be used as a tool to detect exhalations in real world unsupervised inhaler audio signals. K was also calculated to compare the level of agreement between the proposed algorithm and the gold standard method of classification. Taking the classification of the algorithm as one output and the classification of respiratory clinicians as the gold standard output, K was found to be 0.664, indicating substantial agreement between the two classification methods.

5.1.6. Acoustic method of assessing exhalations during inhaler use

In addition to being able to detect exhalations, it would also be beneficial to know if exhalations have the potential to effect drug delivery in a DPI. For this experiment, exhalations occurring at a distance of 5 cm or less, into the DPI mouthpiece or directly at the INCA^{TM} device were classified as significant. It was found that the equation developed to classify a significant exhalation had a sensitivity of 72.22% and a specificity of 85.71% when tested on the validation dataset. Results of this test, in addition to positive predictive value (PPV) and negative predictive value (NPV) are presented in Table 5.5.
In terms of classifying exhalations that occur specifically at 0 cm from the inhaler mouthpiece or with lips sealed around the inhaler mouthpiece, the equation developed had a sensitivity of 88.89% and specificity of 70.73%. For classifying exhalations that occur at 5 cm from the inhaler mouthpiece, the equations developed had a sensitivity of 81.25% and specificity of 88.24%. PPV and NPV for these tests are displayed in Table 5.5. Detailed results can be found in Tables 5.6 – 5.8.

Table 5.5: Assessing significance and location of exhalations during inhaler use.

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Significant Exhalation	72.22	85.71	92.86	54.55
Exhalation at 0 cm/mouthseal	88.89	70.73	40.00	96.67
Exhalation at 5 cm	81.25	88.24	76.47	90.91

Table 5.6: Confusion matrix for a significant exhalation

Significant Exhalation	**Reference POSITIVE**	**Reference NEGATIVE**	**Total**	Sensitivity	**72.22**
Test POSITIVE	26	2	28	Specificity	85.71
Test NEGATIVE	10	12	22	PPV	92.86
Total	36	14		NPV	54.55

Table 5.7: Confusion matrix for an exhalation at 0 cm from the inhaler or with a mouthseal

Exhalation at 0 cm/ MS	**Reference POSITIVE**	**Reference NEGATIVE**	**Total**	Sensitivity	**88.89**
Test POSITIVE	8	12	20	Specificity	70.73
Test NEGATIVE	1	29	30	PPV	40.00
Total	9	41		NPV	96.67

Table 5.8: Confusion matrix for an exhalation at 5 cm from the inhaler

Exhalation at 5 cm	**Reference POSITIVE**	**Reference NEGATIVE**	**Total**	Sensitivity	**81.25**
Test POSITIVE	13	4	17	Specificity	88.24
Test NEGATIVE	3	30	33	PPV	76.47
Total	16	34		NPV	90.91

5.2. Discussion

Several commentators have argued that exhaling into a DPI prior to inhalation has a detrimental impact on the dose available for pulmonary delivery.(71, 76, 81-83) There are very few studies that have been done to clearly define the significance of this effect; nonetheless, exhalation into a DPI has been widely reported as a critical error in the assessment of inhaler technique. In this study, we aimed to show the relationship between factors related to an exhalation and the amount of drug lost or unavailable for delivery during the subsequent inhalation using established *in vitro* methods.

The DUSA apparatus was used to show the delivered dosage uniformity of ten standard doses from a Diskus™ DPI. The results fell within the US Pharmacopoeial specifications: approximately 90% of both salmeterol and fluticasone were recovered from the Diskus™. Our results showed that exhalation into the Diskus™ DPI had a significant effect on the subsequent delivered dose and that the main determining factors were distance of the exhalation from the DPI mouthpiece, flow rate of exhalation and humidity of exhaled air. The most important of these was distance of the exhalation from the mouthpiece. The duration of the exhalation had a negligible effect on drug dispersal, even though it was a statistically significant variable in our regression model. On average, more than 50% of salmeterol and fluticasone were dispersed from the DPI after exhalation from a distance of 0 cm using humid air. At 10 cm, less than 25% of drug was lost.

Experts in the field of inhalers have theorized that the exhalation of humidified air into a DPI causes agglomeration or clumping of the preparation inside the inhaler, subsequently affecting fine particle dose and overall deposition. However this theory has never been proven. We have shown that the relationship between flow rate, distance and duration of exhalation using humidified air is less predictable than that using dry air. Drug agglomeration provides a plausible explanation for our results. Particles that have clumped together may either remain inside the DPI or be emitted as a large mass; this

accounts for the greater variability in total delivered dose seen with humidified air.

To clarify the effect of air humidity, we performed experiments using the Next Generation Cascade Impactor. Our results showed that even though total recovery may remain constant after an exhalation with humid air, the fine particle fraction is almost halved, meaning that most of the dose emitted is deposited in the upper airways.

Clearly, exhalation into a DPI has a significant and measurable negative effect on drug available for delivery during a subsequent inhalation. Our results confirm that this observed patient behaviour is a critical error in inhaler user technique and methods to monitor and address this error are needed. Our group has devised a novel acoustic monitoring device for long term monitoring of inhaler user adherence and technique. In the second part of this study we tested the ability of this acoustic recording device to detect exhalations prior to inhalations and the sensitivity and specificity of our algorithms for estimating the distance of the exhalation from the inhaler mouthpiece.

Our detection algorithm was very accurate at detecting exhalations in unsupervised real world inhaler audio signals in comparison to two expert respiratory clinicians. Its overall accuracy was demonstrated to be 89.2% in detecting exhalations events from non-exhalation events, while its corresponding sensitivity and specificity values were also high. These results are encouraging if such an algorithm is to be used to automatically detect the critical error of exhaling into a DPI.

Furthermore, our calculations based on acoustic power in various frequency bands and mean absolute deviation of the amplitude of the exhalation signal was very sensitive and specific for detecting a significant exhalations and for differentiation of an exhalation at 0 cm from one at 5 cm. Our in vitro studies clearly showed that distance was the single most important factor accounting for drug dispersal or loss from the DPI. Our acoustic device is therefore a

suitable means of not only automatically detecting exhalations but of objectively quantifying the impact exhalations on drug delivery.

The major shortcoming of our study is that it was limited to in vitro techniques; the individual variability in inhaler technique and the confounding factors of biological variation in metabolism, inhalation flow rate, volume and additional errors meant that the impact of exhalations would be difficult to measure accurately in an in vivo pharmacokinetic study. The detection of exhalations and the effect quantification is also limited by the fact that exhalations directed at the INCA^{TM} acoustic device were included in our classification of a significant exhalation. It was impossible to differentiate between an exhalation directly at the mouthpiece and one directed at the INCA^{TM} device because of the location of the device on the inhaler. Further improvements in the device, such as the addition of a second microphone below the inhaler may allow us to filter out exhalations aimed at the device itself.

The current gold standard in assessing inhaler technique is the checklist method. This method is fraught with limitations; it is very subjective and it cannot be used to monitor patients longitudinally. There is also a significant Hawthorne effect where patients change their behaviour because they know they are being assessed. We need to strive towards more objective methods for the assessment of inhaler technique and methods that allow patients' inhaler technique to be monitored continuously. Recent advances in acoustic analysis and signal processing mean that it is now possible to use the sound profile of an exhalation detected during inhaler use as a surrogate measure of the amount of drug unavailable for subsequent delivery. This provides a way for monitoring patterns of use in a rolling fashion and making necessary adjustments to technique.

CHAPTER 6: Does orientation of the Diskus™ inhaler affect available dose for delivery?

6.1. Results

Actuating a dose or a blister while the Diskus™ was held still at 0°, 45° or 90° did not remove detectable amounts of active drug from the inhaler (Table 6.1). When the device was held at 0°, a dose actuated and the device subsequently tilted to the 90° or vertical position, approximately 5 mcg of salbutamol or 2.5% of the available dose was removed from the Diskus™. Tapping the inhaler after actuating a dose in the 90° position similarly did not significantly affect the amount of drug removed (average of 4.8% of available dose and maximum of 8.4%).

Only when the Diskus™ was held at the 90° position after dose actuation and shaken was significant amount of drug removed (54% of available dose removed with a range of 39.4% to 62.3%). Experimental results are shown in Figure 6.1.

Table 6.1: Amount of drug (mcg) removed from the Diskus™ inhaler when held different positions.

	Drug removed (mcg)					
Diskus™ position	**0° Still**	**45° Still**	**90° Still**	**90° Tilt**	**90° Shaken**	**90° Tapped**
Run 1	<0.5	<0.5	<0.5	<0.5	123.8	4.0
Run 2	<0.5	<0.5	<0.5	11.0	123.2	4.6
Run 3	<0.5	<0.5	<0.5	5.1	124.6	12.5
Run 4	<0.5	<0.5	<0.5	8.9	92.7	9.6
Run 5	<0.5	<0.5	<0.5	<0.5	78.8	16.7
Mean	<0.5	<0.5	<0.5	5.1	108.6	9.5
Standard Deviation	-	-	-	4.9	21.5	5.4

6.2. Discussion

The Diskus™ manufacturer and all checklists reviewed for correct Diskus™ technique recommend holding the Diskus™ either horizontal or upward after dose actuation or blistering and holding the Diskus™ in the horizontal position during inhalation. There are no in vitro or in vivo studies available that show the impact of Diskus™ position on available dose, although it is likely that the manufacturer did testing of this kind during device development and validation.

This study showed that holding the Diskus™ at a 45° angle to the horizontal position or in a vertical position with the mouthpiece facing down after blistering had an insignificant effect on the amount of drug removed from the device. This evidence contradicts current thinking on correct Diskus™ inhaler technique and manufacturer's recommendations. Shaking the device while it was held in the downward position was, however, clearly effective at removing over half the available drug from the inhaler and this manouvre is not uncommon among Diskus™ users, who are used to shaking their pMDI inhaler prior to dosing and inhalation. Figure 3.8 shows a visual example of a patient shaking his/ her inhaler after blistering a dose.

While acoustic monitoring is not able to detect the Diskus™ position in space, it can detect when the device is shaken or tapped. From the above experiments, it is clear that these are the only two scenarios where significant amounts of active drug are removed from the inhaler. Consideration was given to incorporating a digital inclinometer with or without an accelerometer into the INCA™ device to better assess Diskus™ position in space and movement, based on the above results, the likely benefits do not outweight the cost of this technology.

The method used for estimating drug removed although effective, is a crude surrogate measure. Further studies on the effect of Diskus™ position on drug delivery should include cascade impactor studies to determine whether angle changes the flow dynamics and pharmacokinetic studies, which will better approximate real world scenarios. Use of a clamp stand in this study is unlikely

to capture the variability in position and movement during Diskus™ use in a patient cohort.

CHAPTER 7: Impact of breath holding on drug delivery

7.1. Results

All subjects recruited were healthy with no underlying respiratory conditions. The mean age was 25.3 ± 4.8 years and five of the seven subjects were male (71%).

Table 7.1 shows the average serum salbutamol concentration at different dosing time-points. Dose 1 Time 0 represents baseline concentration and all subjects had salbutamol levels below the lower limit of quantification of the assay prior to starting the study. After taking the first dose, there was a trend towards a smaller increaser in serum salbutamol concentration when breath hold duration was 4 seconds compared to 10 seconds; however, this difference was not statistically significant.

There was a statistically significant 29% reduction in trough salbutamol concentration when subjects had only a 4 second breath hold duration compared to correct inhaler technique with a 10 second breath hold. There was also a 22% lower rise in salbutamol concentration from the dose 6 trough level when using a 4 second breath hold. These trends are highlighted in Figure 7.1.

All subjects except for subject 3 had a lower pre-dose 6 trough concentration with a 4 second breath hold and all except subjects 1 and 2 had a smaller absolute rise from pre-dose 6 trough levels with a 4 second breath hold (Table 7.2).

Table 7.1: Average serum salbutamol concentration at different dosing time-points and different breath hold durations, with absolute and relative differences and p-values calculated from a one-sided t-test.

Mean salbutamol concentration (ng/ml) at:	Breath hold duration		Absolute difference	Relative difference (%)	p-value (1-sided)
	10 seconds	4 seconds			
Dose 1 Time 0	< 0.04	< 0.04	0.00	0.0	0.500
Dose 1 Time 25	0.50	0.46	- 0.04	- 8.0	0.232
Dose 6 Time 0	0.86	0.61	- 0.25	- 29.0	0.024
Dose 6 Time 25	1.39	1.09	- 0.30	- 21.6	0.049

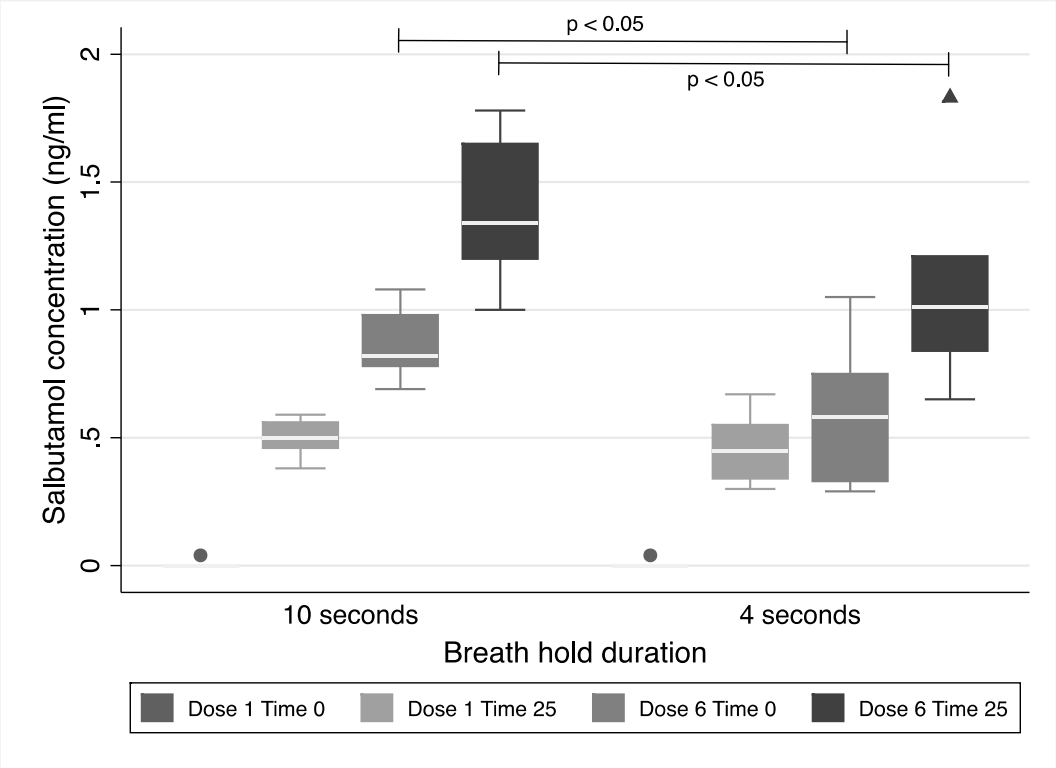

Figure 7.1: Boxplot showing mean salbutamol concentrations (n=7) at different time points and at two different breath hold durations (10 s and 4 s). There was a significant lower Dose 6 Time 0 trough level and the Dose 6 Time 25 peak level when breath-hold duration was 4s.

Table 7.2: Salbutamol concentration levels at different time points for subjects 1 to 7 at two different breath hold durations (10s and 4s).

ID	Age	Sex	Dose 1 Time 0	Dose 1 Time 25	Dose 1 Peak - Trough	Dose 6 Time 0	Dose 6 Time 25	Dose 6 Peak - Trough	Breath hold duration (s)
			Salbutamol concentration (ng/ml)						
1	30	M	0	0.5	0.5	0.69	1	0.31	10
1	30	M	0	0.42	0.42	0.58	1.01	0.43	4
2	20	M	0	0.49	0.49	0.82	1.2	0.38	10
2	20	M	0	0.67	0.67	0.29	0.84	0.55	4
3	22	F	0	0.54	0.54	0.86	1.78	0.92	10
3	22	F	0	0.55	0.55	1.05	1.83	0.78	4
4	20	M	0	0.56	0.56	0.78	1.24	0.46	10
4	20	M	0	0.45	0.45	0.33	0.65	0.32	4
5	27	M	0	0.46	0.46	0.98	1.65	0.67	10
5	27	M	0	0.34	0.34	0.72	1.21	0.59	4
6	26	F	0	0.38	0.38	0.79	1.34	0.55	10
6	26	F	0	0.3	0.3	0.52	0.98	0.38	4
7	32	M	0	0.59	0.55	1.08	1.54	0.46	10
7	32	M	0	0.5	0.46	0.75	1.13	0.38	4

7.2. Discussion

Breath holding after inhalation enables particles delivered to the smaller airways to be deposited in that region, instead of being exhaled.(96) Most studies on the effect of breath holding on DPI drug delivery have used in silico methods. This is the first pharmacokinetic study on the impact of breath holding duration when using a Diskus™ DPI. Again, salbutamol was chosen as the active drug due to its short half-life and thus, short washout period, its safety profile and the relatively high serum concentrations after inhalation.

The results above show that reducing the breath hold duration from 10 seconds to 4 seconds causes an almost 30% reduction in steady state trough concentrations and an approximate 20% reduction in peak levels when a dose is taken at steady state. However, it is noteworthy that this pattern was not observed in all patients. A possible explanation for this is the inter- and intra-individual variability in bioavailability and features of inhaler technique from dose to dose that cannot be detected by the INCA™ device.

Compared to the effect of low PIFR on drug delivery, the effect of low breath hold duration is less significant. Since this study did not look at any pharmacodynamic measures, the possible downstream effect of a 20-30% reduction in serum concentration levels remains to be determined. This 20-30% change is likely to be more clinically significant when short breath hold duration is combined with other technique errors, such as low peak flow rate.

The INCA™ device readily allows determination of multiple inhalations and coughing during inhaler use (see Figures 3.4 and 3.5), which are both associated with a subsequent shortening of breath hold duration. One limitation of the INCA™ device in monitoring breath hold duration is that some patients close their inhaler prior to exhalation after inhaling a dose. Thus, breath hold duration in this circumstance can only be measured from the time of inhalation to the time the device is closed. This iteration of the INCA™ device focuses mainly on detecting multiple inhalations and coughing and for

patients with an audible exhalation after inhaling a dose, the INCA^{TM} device can also be used to accurately quantify breath hold duration.

Ideally, this study should be repeated at breath hold durations of 1 second and 2 second to establish whether such short durations have an even greater effect on drug delivery. This study focused on only two durations since each breath-hold duration studied added four venipunctures per subject; further repetitions were limited by subject willingness to participate.

CHAPTER 8: Impact of missed doses on steady state trough and peak levels

8.1. Results

Based on first-order kinetics and a half-life of salbutamol of 6 hours, pharmacokinetic curves were generated to mimic regular 6 hourly dosing for 6 doses (Figure 8.1) and when doses 3 and 4 are missed (Figure 8.2). Based on these curves, pre-dose trough levels were calculated. The expected mean trough level when doses 3 and 4 are missed should theoretically be 64% of the mean trough level when all doses are taken as specified (Figure 8.3).

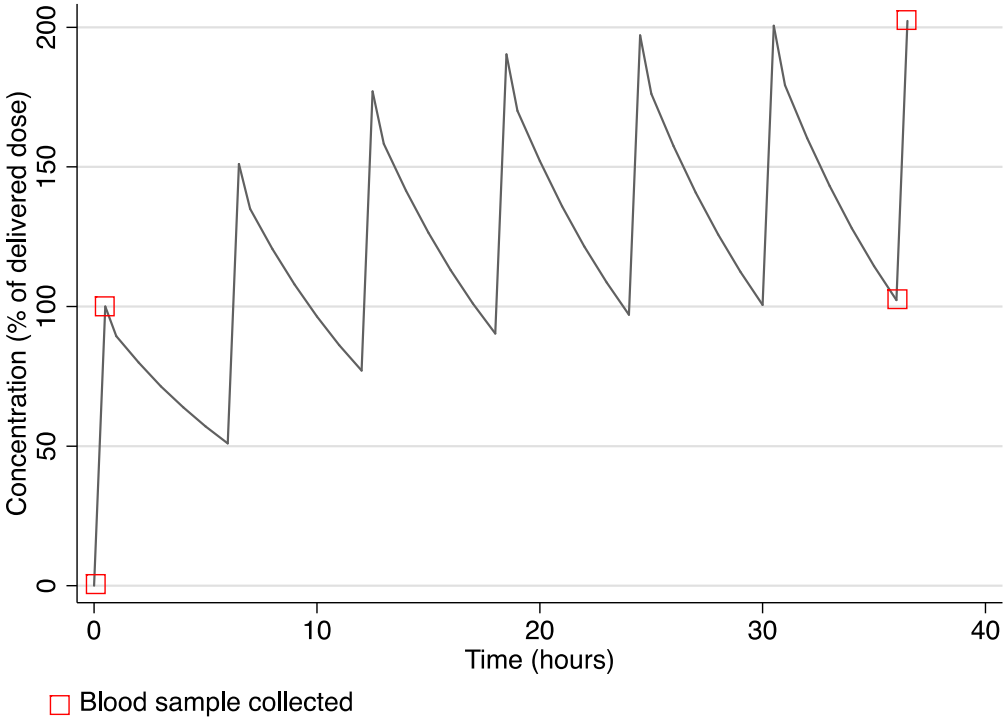

Figure 8.1: Simulated pharmacokinetic profile when all doses are taken at 6 hourly intervals.

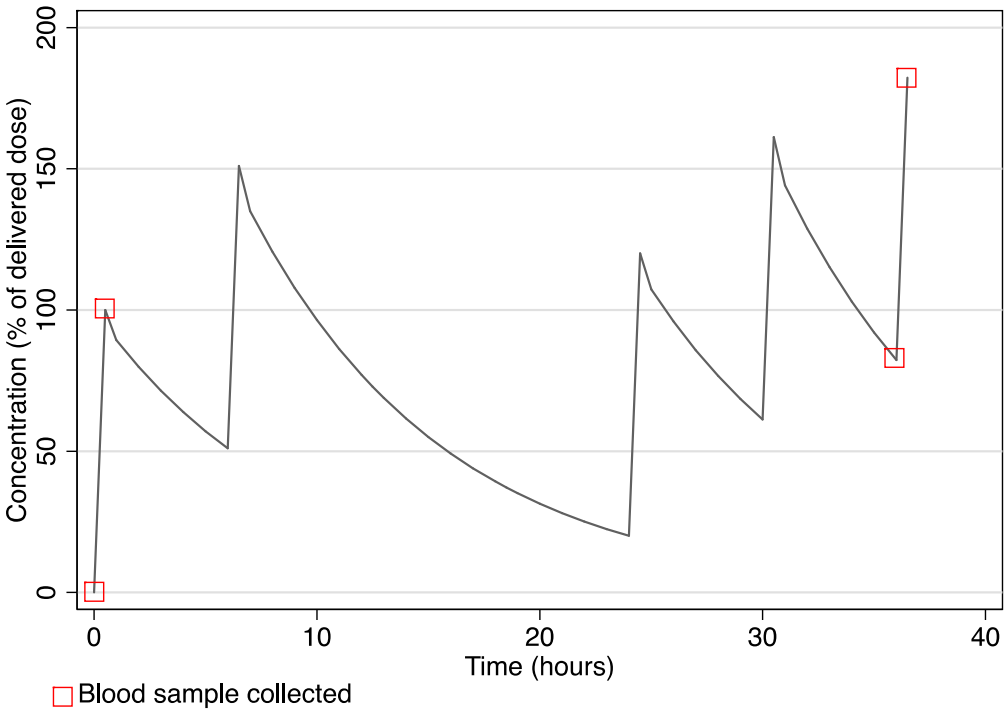

Figure 8.2: Simulated pharmacokinetic profile when doses are taken at 6 hourly intervals with the exception of doses 3 and 4.

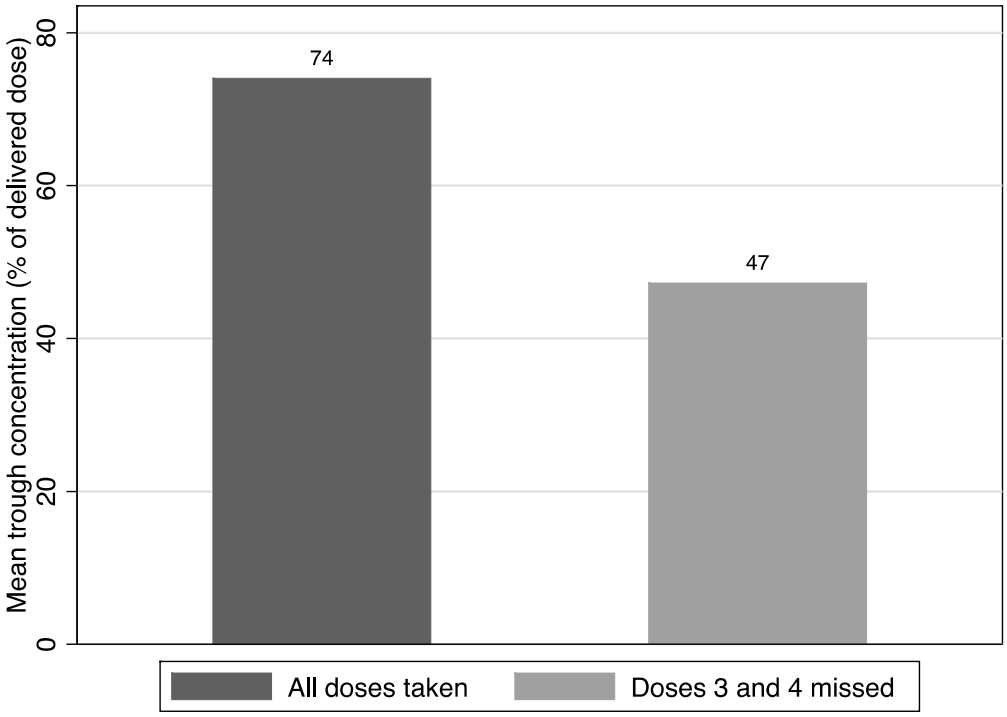

Figure 8.3: Bar graph showing mean trough concentration expected when all doses are taken and when doses 3 and 4 are missed.

Four healthy volunteers were enrolled with a mean age of 20.3 years. The salbutamol concentrations during the control and missed dose phases are shown in Table 8.1 and Figure 8.4.

Table 8.1: Salbutamol concentrations at different time points in the control phase (all doses taken) and the missed doses phase (doses 3 and 4 missed).

			Control				Missed doses			
ID	**Age**	**Sex**	**Dose 1 Time 0**	**Dose 1 Time 25**	**Dose 6 Time 0**	**Dose 6 Time 25**	**Dose 1 Time 0**	**Dose 1 Time 25**	**Dose 6 Time 0**	**Dose 6 Time 25**
1	19	M	<0.04	0.62	0.68	1.03	<0.04	0.51	0.53	1.26
2	24	F	<0.04	0.82	0.86	1.7	<0.04	0.49	0.66	1.68
3	19	M	<0.04	0.35	0.84	0.72	<0.04	0.34	0.48	0.75
4	19	M	<0.04	0.58	1.03	0.67	<0.04	0.31	0.54	0.98
Mean	**20.3**	**3:1**	**<0.04**	**0.59**	**0.85**	**1.03**	**<0.04**	**0.41**	**0.55**	**1.17**

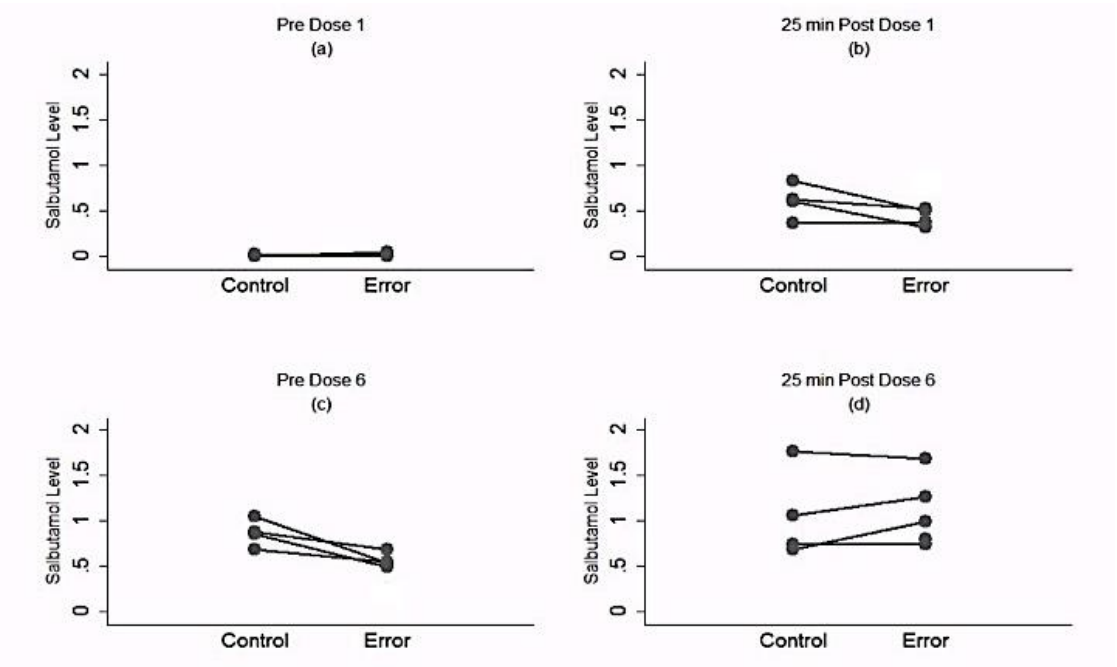

Figure 8.4: Connected dotplots of salbutamol concentration by subject for control phase and missed doses phase at time points: (a) Dose 1 time 0, (b) Dose 1 time 25, (c) Dose 6 time 0, and (d) Dose 6 time 25.

There was no significant difference in concentration levels pre- and post-dose 1 but there was a statistically significant 64% difference in pre-dose 6 trough concentration (control: 0.85 ng/ml and missed doses: 0.55 ng/ml, $p<0.001$). There was no significant difference between post-dose 6 peak levels.

8.2. Discussion

Based on the theoretical pharmacokinetic models, the pre-dose 6 trough concentration when doses 3 and 4 are missed should be approximately 80% of the level when all 6 doses are taken with the same technique, that is, a 20% absolute reduction. The results from the in vivo study showed that the two missed doses caused a significant 35% absolute reduction in trough concentration. Even with a small sample size, this effect was statistically significant. As expected, there was no difference in the peak level achieved after dose 1 since this is dependent solely on inhaler technique. There was a trend towards a higher post-dose 6 peak level but this was not statistically significant and could possibly be explained by variations in inhaler technique when taking dose 6.

Importantly, our theoretical pharmacokinetic model predicted a 36% reduction in mean trough concentration when doses 3 and 4 are missed. There was also a greater variability in trough concentrations over time with missed doses. From the in vivo studies, the effect size of missed doses appears to be less than that of low PIFR but greater than that of breath hold duration. Again, since pharmacodynamic parameters were not recorded, it is difficult to draw conclusions about the clinical relevance of a 35% reduction in trough concentrations. However, it is noteworthy that while most investigators focus on temporal adherence in clinical studies, technique factors potentially have a greater impact on drug delivery since the same technique errors are performed with most doses leading to a cumulative effect.

The small sample size of this study limits the generalizability of the results but does highlight similar trends to those predicted. This study only looked at the effect of missing doses 3 and 4; it is likely that other combinations of missed doses may have more significant effects on pre-dose 6 trough levels but the effects will average out when measuring mean trough concentrations.

This study used a salbutamol half-life of 6 hours. The mean elimination half-life of salbutamol after oral administration is approximately 5.7 ± 1.4 hours and

after inhalation is 6.1 ± 2.1 hours.(35) The wide range of half-lives reflects inter-individual variability in metabolism as well as technique; inhalation technique appears to be significant since the standard deviation for inhalation is wider than that for oral administration. Due to this wide range of half-lives, the generalizability of our theoretical pharmacokinetic model is also questionable. Future pharmacokinetic studies should obtain a complete pharmacokinetic profile to allow calculation of the drug half-life for every subject. These values can then be used in individualized models of salbutamol dosing.

CHAPTER 9: Development and validation of an algorithm for combining time and technique of inhaler use into a single metric

9.1. Results

9.1.1. Algorithm development

Based on the evidence in prior chapters, Diskus™ inhaler technique errors were classified as either critical or non-critical (Table 9.1).

A first-order pharmacokinetic model was used with a functional half-life of 12 hours (to mimic twice-daily dosing of the salmeterol/ fluticasone inhaler). Bolus doses were assigned using the date- and time- stamped data from the INCA™ device. The fine particle fraction assigned to each bolus was based on the estimated peak inspiratory flow rate and the presence or absence of technique errors (Table 9.2). Where multiple errors occurred on the same dose, the assigned dose fraction attached to the errors were multiplied to arrive at the final FPF assigned to that dose. The concentrations used represent small airways concentration of the active drug.

Table 9.1: Classification of Diskus™ technique errors as critical or non-critical. All errors can be detected by the INCA™ device unless stated otherwise.

Step	Correct step	Possible technique errors	Is error critical?
1	Push the outer cover as far as possible before the inhalation	Failure to open the outer cover Incomplete opening of outer cover	Yes
2	Slide the lever until the ”click” sound to actuate dose	Failure to slide the lever until the “click” sound	Yes
3	Keep the mouthpiece horizontal or in upward position	Holding the Diskus™ inhaler with the mouthpiece facing downward	No – not assessed by INCA
4	Exhale into the room and away from the mouthpiece after loading	Exhalation into the device mouthpiece	Yes
5	Slowly and completely exhale out to residual volume (to empty the lungs)	(1) No exhalation or insufficient exhalation (2) Forced and fast exhalation	Probable – not assessed
6	Tilt head back (hyperextend) slightly and keep the device horizontal during inhalation	Lowering one’s head or holding the mouthpiece upward during inhalation	Probable – not assessed by INCA
7	Place teeth over the mouthpiece with lips positioned around it deeply (over tongue) and securely (sealing lip)	(1) Lips surround the mouthpiece shallowly against teeth or tongue (2) Lips are not sealed around the mouthpiece during inhalation	Probable – not assessed by INCA
8	Inhale forcefully from the beginning, slowly (for > 2–3 sec), deeply, uniformly, and continuously inhale during the inspiratory phase until the lungs are full	(1) Gradual increase in the speed of inhalation (2) Fast and extremely forceful inhalation (3) Prematurely stop inhaling (not inhaling to total lung capacity) or inhaling twice or more during the inspiratory phase of the breathing cycle	Yes
9	At the end of inhalation remove the inhaler from the mouth and close the lips	Not removing the inhaler from the mouth at the end of inhalation	No - not assessed by INCA
10	Hold breath for > 5 sec (optimally for 10 sec) after inhalation (an objective measurement performed using a stopwatch)	Not holding breath or holding breath for < 5 sec	Yes
11	Exhale slowly through the nose and away from mouthpiece	(1) Breathing out rapidly from the mouth after holding breath (2) Exhaling into the mouthpiece	Unlikely – not assessed
12	Recover the lever and the outer cover	Not closing the lever and the outer cover	No
13	Rinse one’s mouth out after inhaling and do not swallow the rinsing water	(1) Failure to rinse one’s mouth (2) Swallowing the rinsing water	Significant for adverse events – not assessed

Table 9.2: Breakdown of factors that were included in algorithm to generate the fine particle fraction assigned to every dose.

Technique step	Error	Dose assigned
Open inhaler	N	100%
	Y	0%
Actuate dose lever	N	100%
	Y	0%
Exhale away from the mouthpiece	N	100%
	Y	50%
Inhaler shaking/ tapping after dose actuation	N	100%
	Y	50%
Inhale deeply and forcefully		Salmeterol_FPF (%)=0.176*PIFR + 0.627*InhaleDuration + 5.915 Fluticasone_FPF (%)=0.178*PIFR + 0.640*InhaleDuration + 5.538
Breath holding duration > 10 seconds	N	100%
	Y	75%
Multiple inhalations or coughing after inhalation (<10 second breath hold)	N	100%
	Y	75%

The Stata algorithm code developed is shown in Box 9.1. The trough concentrations were calculated using the following equations:

$$SALM_{trough} = SALM_{trough}[n-1] + SALM_{FPF}[n] * \mathrm{e}^{-0.693* \ \frac{Time\ \Delta}{12}} \quad (9.1)$$

$$FLUT_{trough} = FLUT_{trough}[n-1] + FLUT_{FPF}[n] * \mathrm{e}^{-0.693* \ \frac{Time\ \Delta}{12}} \quad (9.2)$$

Figure 9.1 represents the dosing profile simulated by the algorithm when doses are taken every 12 hours with correct technique. This contrasts with the dosing profile simulated with missed doses (absence of a blue bolus dots at 12 hour intervals in Figure 9.2), overdoses (spike in trough concentration green line in Figure 9.2) or when technique errors are made and doses are missed

(FPF% assigned to a blue dot and absence of blue dots at 12 hour intervals in Figure 9.3). The trough concentration is significantly different between the two simulated patient profiles.

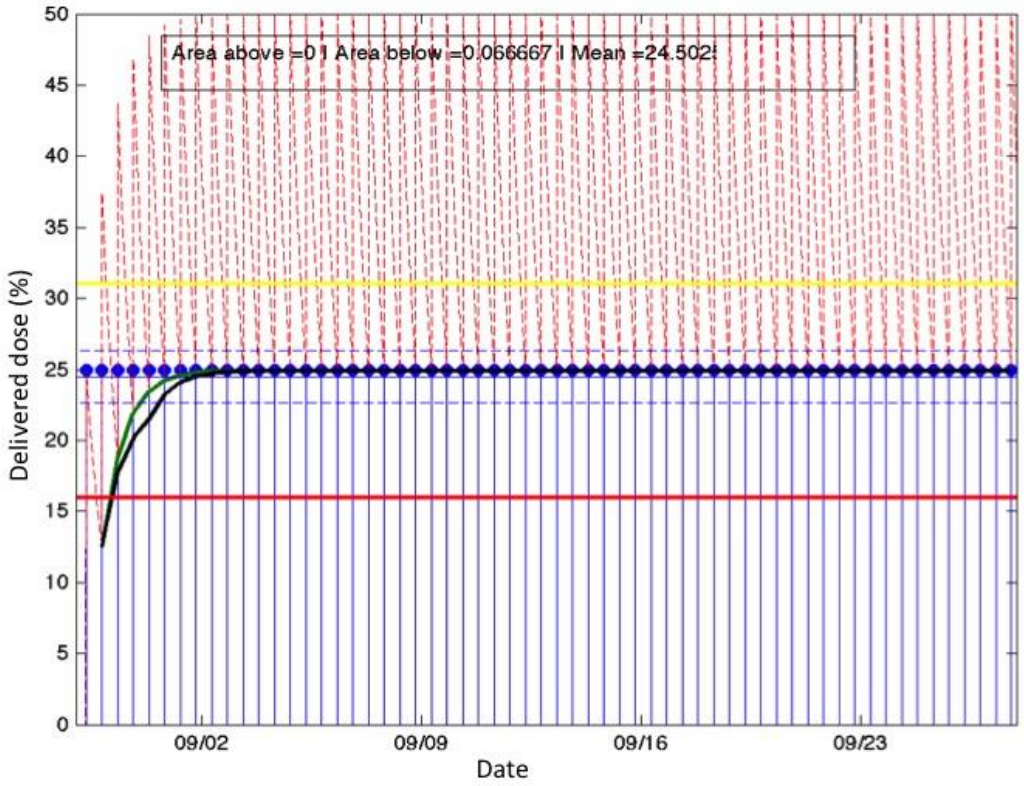

Figure 9.1: Pharmacokinetic dosing profile expected with 12 hourly dosing interval and correct inhaler technique. The green line represents the expected pre-dose trough small airway concentration, the yellow line represents the minimum toxic concentration predicted from over-dosing, the red line represents the minimum effective concentration using a peak inspiratory flow rate of 30 l/min, the blue dots represent bolus doses and the red dashed lines represent change in concentration over time. The area above is calculated by integrating the area between the green trough curve and the yellow line. The area below is calculated by integrating the area between the green trough curve and red line. “Mean” represents the average pre-dose trough concentration.

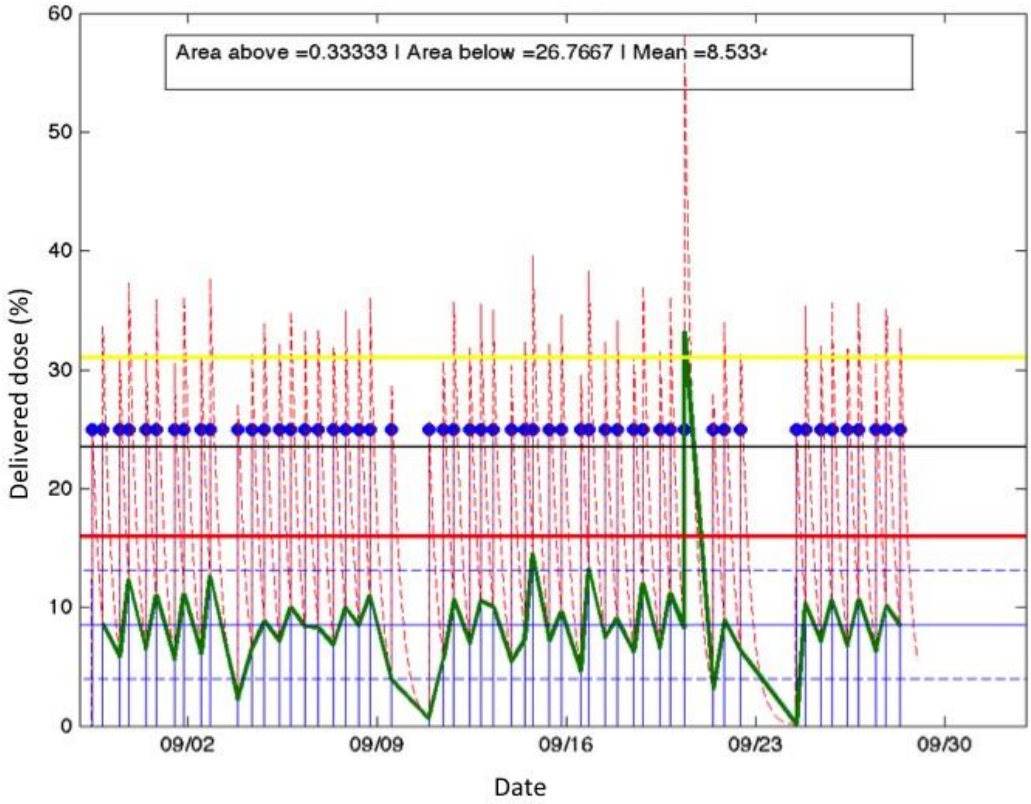

Figure 9.2: Pharmacokinetic dosing profile expected with 12 hourly dosing interval and correct inhaler technique when doses are missed (absence of blue dots) or extra doses are taken (green line above yellow line). The green line represents the expected pre-dose trough small airway concentration, the yellow line represents the minimum toxic concentration predicted from over-dosing, the red line represents the minimum effective concentration using a peak inspiratory flow rate of 30 l/min, the blue dots represent bolus doses and the red dashed lines represent change in concentration over time. The area above is calculated by integrating the area between the green trough curve and the yellow line. The area below is calculated by integrating the area between the green trough curve and red line. “Mean” represents the average pre-dose trough concentration.

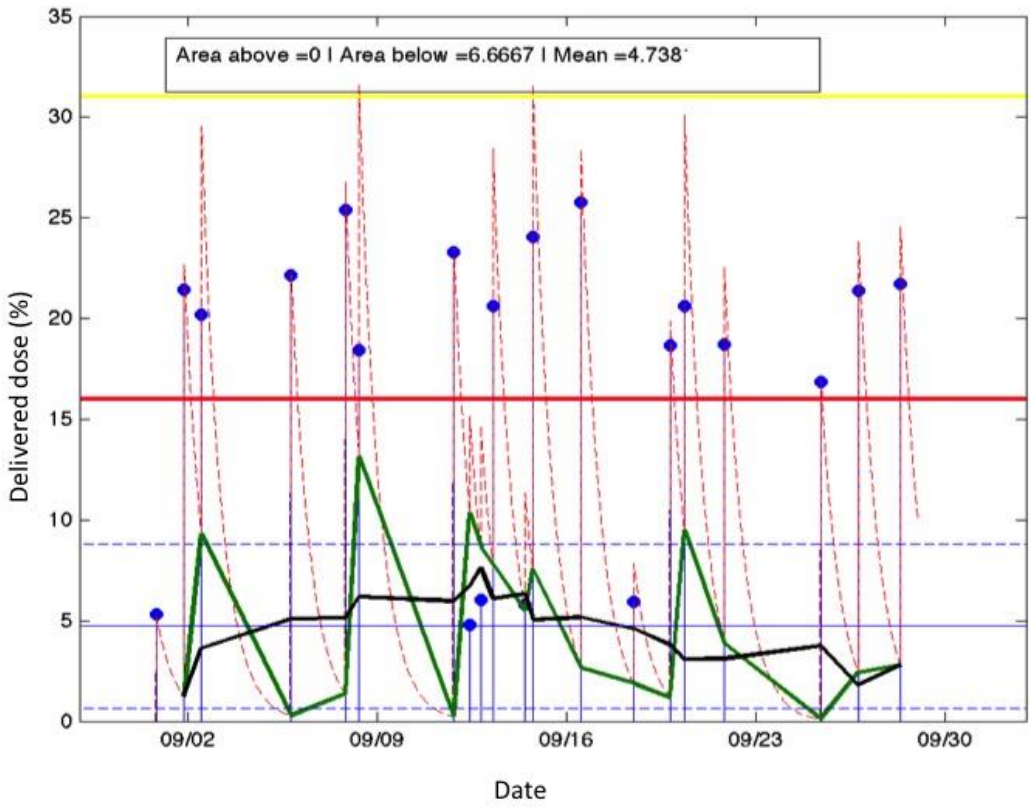

Figure 9.3: Pharmacokinetic dosing profile expected with 12 hourly dosing interval and technique errors (variation in delivered dose %) when doses are missed (absence of blue dots). The green line represents the expected pre-dose trough small airway concentration, the yellow line represents the minimum toxic concentration predicted from over-dosing, the red line represents the minimum effective concentration using a peak inspiratory flow rate of 30 l/min, the blue dots represent bolus doses and the red dashed lines represent change in concentration over time. The area above is calculated by integrating the area between the green trough curve and the yellow line. The area below is calculated by integrating the area between the green trough curve and red line. “Mean” represents the average pre-dose trough concentration.

Box 9.1: Stata code for dosing algorithm

```
qui foreach y in 1 2 3 {
clear all
scalar indir= "/Users/~/Desktop/"
scalar outdir= "/Users/~/Desktop/"
cd "`=indir'"
import excel `y'.xlsx, sheet("MS") firstrow clear
gen date2=.
capture confirm string variable Date
	if !_rc {
		replace date2=date(Date, "DM20Y")
                                                        }
	else {
		replace date2=Date
                                                        }
format date2 %td
format Time %tcHH:MM:SS
gen InhaleDuration=InhaleStartTime
gen timed_doses=ORClass>0
gen combined_doses=ORClass==1
gen technique_errors=(ORClass==2)*-1
gen SALM_FPF=0.1755314*PIFR + 0.6265714*InhaleDuration + 5.915075
gen FLUT_FPF=0.1778574*PIFR + 0.6396681*InhaleDuration + 5.538353
gen SALM_UAD= 0.1044824*PIFR + 64.04375
gen FLUT_UAD=0.0460495*PIFR + 63.44969
capture confirm string variable ORError
	if !_rc {
		replace SALM_FPF = SALM_FPF*0.5 if strpos(ORError, "2")
		replace FLUT_FPF = FLUT_FPF*0.5 if strpos(ORError, "2")
		replace SALM_FPF = SALM_FPF*0.5 if strpos(ORError, "3")
		replace FLUT_FPF = FLUT_FPF*0.5 if strpos(ORError, "3")
		replace SALM_FPF = SALM_FPF*0.75 if strpos(ORError, "4")
		replace FLUT_FPF = FLUT_FPF*0.75 if strpos(ORError, "4")
		replace SALM_FPF = SALM_FPF*0.75 if strpos(ORError, "5")
		replace FLUT_FPF = FLUT_FPF*0.75 if strpos(ORError, "5")
		replace SALM_FPF = SALM_FPF*0 if ORClass == 0
		replace FLUT_FPF = SALM_FPF*0 if ORClass == 0
		replace SALM_UAD = SALM_UAD*0 if ORClass == 0
		replace FLUT_UAD = SALM_UAD*0 if ORClass == 0
	}
	else {
		replace SALM_FPF = SALM_FPF*0.25 if ORError == 2
		replace FLUT_FPF = FLUT_FPF*0.25 if ORError == 2
		replace SALM_FPF = SALM_FPF*0.25 if ORError == 3
		replace FLUT_FPF = FLUT_FPF*0.25 if ORError == 3
		replace SALM_FPF = SALM_FPF*0.5 if ORError == 4
		replace FLUT_FPF = FLUT_FPF*0.5 if ORError == 4
		replace SALM_FPF = SALM_FPF*0.75 if ORError == 5
		replace FLUT_FPF = FLUT_FPF*0.75 if ORError == 5
		replace SALM_FPF = SALM_FPF*0 if ORClass == 0
		replace FLUT_FPF = SALM_FPF*0 if ORClass == 0
		replace SALM_UAD = SALM_UAD*0 if ORClass == 0
		replace FLUT_UAD = SALM_UAD*0 if ORClass == 0
	}
keep date2 Time VisitNo SALM_FPF FLUT_FPF SALM_UAD FLUT_UAD
order date2, before(Time)
drop if SALM_FPF==.
drop if date2==.
```

```
replace Time = clock("31dec1899 14:00:00", "DMYhms") if Time==. & date2!=date2[_n-1] &
date2!=[_n+1]
replace Time = clock("31dec1899 08:00:00", "DMYhms") if Time==. & date2!=date2[_n-1] &
date2==date2[_n+1]
replace Time = clock("31dec1899 20:00:00", "DMYhms") if Time==. & date2==date2[_n-1] &
date2!=date2[_n+1]
replace Time = clock("31dec1899 14:00:00", "DMYhms") if Time==. & date2==date2[_n-1] &
date2==date2[_n+1]

gen datetime=date2*24*60*60*1000 +60*365*24*60*60*1000 + 15*24*60*60*1000 + Time
format datetime %tcDD/NN/CCYY_HH:MM:SS
gen deltatime=.
gen SALMtrough=.
by VisitNo, sort: gene id=_n
summarize VisitNo
levelsof VisitNo, local(levels)
qui foreach i of local levels {
replace deltatime=(datetime[_n+1]-datetime)/1000/60/60 if VisitNo==`i'
summarize VisitNo if VisitNo==`i'
replace deltatime=. if id==`r(N)' & VisitNo==`i'
replace SALMtrough=(SALM_FPF)*exp(-0.693*deltatime/12) if id==1
forval x=2/`r(N)' {
replace SALMtrough=(SALMtrough[_n-1]+SALM_FPF)*exp(-0.693*deltatime/12) if id==`x'
}
}
gen SALMtrough2=SALMtrough/23.5*100
replace SALMtrough2=100 - (SALMtrough2-100) if SALMtrough2>100
replace SALMtrough2=0 if SALMtrough2<0
gen Avgtrough=.
gen SDtrough=.
gen mintrough=.
gen maxtrough=.
summarize VisitNo
levelsof VisitNo, local(levels)
qui foreach i of local levels {
summarize SALMtrough2 if VisitNo==`i'
replace Avgtrough=`r(mean)' if VisitNo==`i'
replace SDtrough=`r(sd)' if VisitNo==`i'
replace mintrough=`r(min)' if VisitNo==`i'
replace maxtrough=`r(max)' if VisitNo==`i'
}
cd "`=outdir'"
export excel using `y'.xlsx, sheetreplace sheet("Trough_Data_Act_SALM") firstrow(variables)
collapse Avgtrough SDtrough mintrough maxtrough, by(VisitNo)
export excel using `y'.xlsx, sheetreplace sheet("Trough_Data_Summary_Act_SALM")
firstrow(variables)
*outsheet date2 Time SALM_FPF using "`y'SALM_Trough_Data_Actual_Overall.txt", replace

import excel `y'.xlsx, sheet("MS") firstrow clear
gen date2=.
capture confirm string variable Date
        if !_rc {
                replace date2=date(Date, "DM20Y")
                                                }
        else {
                replace date2=Date
                                                }
format date2 %td
format Time %tcHH:MM:SS
```

```
gen InhaleDuration=InhaleStartTime
gen timed_doses=ORClass>0
gen combined_doses=ORClass==1
gen technique_errors=(ORClass==2)*-1
gen SALM_FPF=23.5 if ORClass>0
gen FLUT_FPF=23.5 if ORClass>0
keep date2 Time VisitNo SALM_FPF FLUT_FPF
order date2, before(Time)
drop if SALM_FPF==.
drop if date2==.
replace Time = clock("31dec1899 14:00:00", "DMYhms") if Time==. & date2!=date2[_n-1] &
date2!=[_n+1]
replace Time = clock("31dec1899 08:00:00", "DMYhms") if Time==. & date2!=date2[_n-1] &
date2==date2[_n+1]
replace Time = clock("31dec1899 20:00:00", "DMYhms") if Time==. & date2==date2[_n-1] &
date2!=date2[_n+1]
replace Time = clock("31dec1899 14:00:00", "DMYhms") if Time==. & date2==date2[_n-1] &
date2==date2[_n+1]

gen datetime=date2*24*60*60*1000 +60*365*24*60*60*1000 + 15*24*60*60*1000 + Time
format datetime %tcDD/NN/CCYY_HH:MM:SS
gen deltatime=.
gen SALMtrough=.
by VisitNo, sort: gene id=_n
summarize VisitNo
levelsof VisitNo, local(levels)
qui foreach i of local levels {
replace deltatime=(datetime[_n+1]-datetime)/1000/60/60 if VisitNo==`i'
summarize VisitNo if VisitNo==`i'
replace deltatime=. if id==`r(N)' & VisitNo==`i'
replace SALMtrough=(SALM_FPF)*exp(-0.693*deltatime/12) if id==1
forval x=2/`r(N)' {
replace SALMtrough=(SALMtrough[_n-1]+SALM_FPF)*exp(-0.693*deltatime/12) if id==`x'
}
}
gen SALMtrough2=SALMtrough/23.5*100
replace SALMtrough2=100 - (SALMtrough2-100) if SALMtrough2>100
replace SALMtrough2=0 if SALMtrough2<0
gen Avgtrough=.
gen SDtrough=.
gen mintrough=.
gen maxtrough=.
summarize VisitNo
levelsof VisitNo, local(levels)
qui foreach i of local levels {
summarize SALMtrough2 if VisitNo==`i'
replace Avgtrough=`r(mean)' if VisitNo==`i'
replace SDtrough=`r(sd)' if VisitNo==`i'
replace mintrough=`r(min)' if VisitNo==`i'
replace maxtrough=`r(max)' if VisitNo==`i'
}
cd "`=outdir'"
export excel using `y'.xlsx, sheetreplace sheet("Trough_Data_Att_SALM") firstrow(variables)
collapse Avgtrough SDtrough mintrough maxtrough, by(VisitNo)
export excel using `y'.xlsx, sheetreplace sheet("Trough_Data_Summary_Att_SALM")
firstrow(variables)
*outsheet date2 Time SALM_FPF using "`y'SALM_Trough_Data_Actual_Overall.txt", replace
}
```

9.1.2. Algorithm performance in a patient cohort.

The characteristics and demographics of the 20 patients studied are presented in Table 9.3. Half of the study population were asthmatics and half had an underlying diagnosis of COPD.

Table 9.3: Demographics, primary respiratory diagnosis and smoking status of patient cohort.

Demographic		**Mean (SD) or count (%)**
Age		54.8 (19.5) years
Gender	Male	8 (40)
	Female	12 (60)
Diagnosis	Asthma	10 (50)
	COPD	10 (50)
Smoking status	Non-smoker	5 (25)
	Ex-smoker	7 (35)
	Current smoker	8 (40)

Table 9.4 and Figures 9.1 through 9.20 show tabular and graphical representations of the temporal and technique adherence of all 20 subjects over the month of monitoring. There was significant variation in both temporal and technique adherence. Some patients had very erratic dosing with both over dosing and under dosing, others had predominantly under dosing and others had significant numbers of technique errors (three patients had all or almost all doses with technique errors).

Table 9.4: Breakdown of different adherence rates generated by algorithm.

			Timing only					Timing and technique				
Subject ID	Dose counter rate (%)	INCA™ dose Rate (%)	AATR (au)	AUTR (au)	Mean delivered dose (% available dose)	Delivered dose CV (%)	Attempted Rate (%)	AATR (au)	AUTR (au)	Mean delivered dose (% available dose)	Delivered dose CV (%)	Actual Rate (%)
1	**88.2**	**92.1**	0.9	8.6	18.9	32.2	**80.3**	0.0	14.8	13.9	38.1	**59.2**
2	**178.6**	**82.1**	2.2	6.6	20.5	30.6	**87.3**	0.0	14.6	13.8	31.3	**58.7**
3	**137.5**	**92.5**	0.1	4.6	20.8	24.1	**88.5**	0.0	11.3	16.8	25.5	**71.3**
4	**135.3**	**91.2**	3.2	6.9	21.1	38.6	**89.7**	0.0	23.5	8.5	43.5	**36.2**
5	**94.8**	**96.6**	1.6	2.8	22.7	14.5	**96.8**	0.0	11.1	17.3	16.9	**73.8**
6	**88.9**	**88.9**	1.0	6.2	20.3	25.0	**86.5**	0.0	19.3	12.1	25.5	**51.7**
7	**81.3**	**82.8**	1.1	6.6	20.1	28.2	**85.3**	0.0	20.3	11.0	29.8	**47.0**
8	**81.3**	**81.3**	0.6	8.1	18.6	28.5	**79.1**	0.0	19.7	9.2	39.5	**39.3**
9	**90.3**	**96.8**	2.4	5.9	21.4	31.5	**90.9**	0.1	18.4	12.7	29.9	**54.1**
10	**93.8**	**85.9**	1.7	4.5	21.8	23.5	**92.8**	0.0	30.5	5.5	58.0	**23.5**
11	**92.9**	**85.7**	1.0	6.8	19.9	26.6	**84.6**	0.0	17.3	12.9	26.1	**54.8**
12	**90.9**	**90.9**	2.4	5.9	21.5	31.1	**91.3**	0.2	19.5	11.9	35.5	**50.8**
13	**80.6**	**80.6**	0.7	8.3	18.8	28.5	**79.8**	0.0	18.1	12.3	29.3	**52.4**
14	**103.8**	**100.0**	8.8	4.7	25.4	37.9	**107.9**	0.3	19.2	12.2	43.7	**51.9**
15	**84.5**	**86.2**	5.4	8.1	21.8	44.2	**92.8**	0.1	17.3	12.8	48.0	**54.4**
16	**98.1**	**98.1**	2.6	2.8	23.3	16.6	**99.1**	0.0	21.4	12.1	24.3	**51.4**
17	**89.3**	**89.3**	2.6	6.8	21.1	31.1	**89.6**	0.0	20.2	12.1	29.8	**51.6**
18	**70.4**	**70.4**	0.7	9.2	16.8	35.5	**71.6**	0.0	16.3	11.0	36.9	**47.0**
19	**89.3**	**89.3**	1.3	4.8	21.4	21.5	**91.1**	0.0	21.3	11.0	24.1	**46.9**
20	**60.7**	**60.7**	0.1	13.8	13.4	49.4	**56.8**	0.0	22.7	6.6	48.1	**27.9**

AATR: Area between dosing curve and upper limit of therapeutic range; AUTR: Area between dosing curve and lower limit of therapeutic rang; au: arbitrary units.

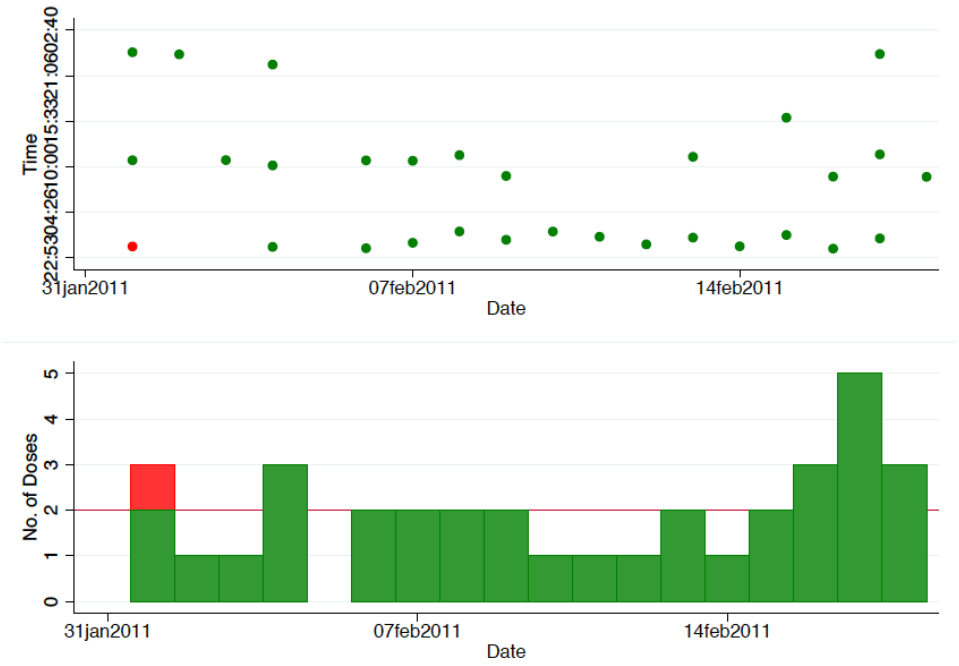

Figure 9.4: Patient 1- Scatterplot showing time of date and time of use. Green dots represent correct technique and red dots represent a technique error. Bar graph shows number of doses taken per day with a target of 2 doses per day (red line). Green bars represent correct technique and red bars represent technique errors. **This patient has periods of over-dosing and missed doses with only one technique error (Class 1).**

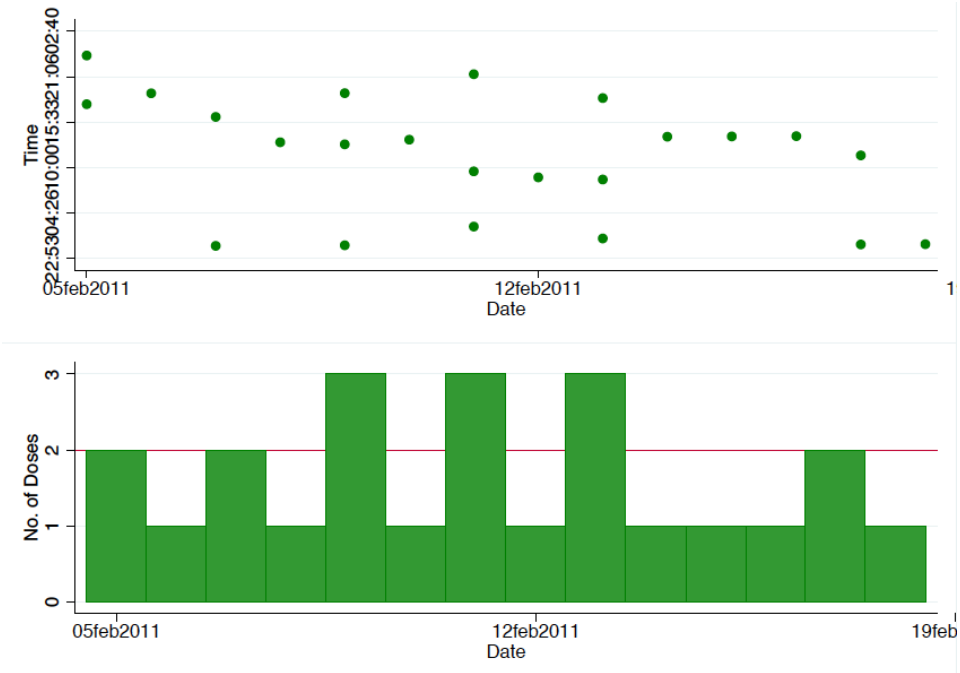

Figure 9.5: Patient 2- Scatterplot showing time of date and time of use. Green dots represent correct technique and red dots represent a technique error. Bar graph shows number of doses taken per day with a target of 2 doses per day (red line). Green bars represent correct technique and red bars represent technique errors. **This patient has no technique errors but erratic dosing with both overdosing and underdosing (Class 1).**

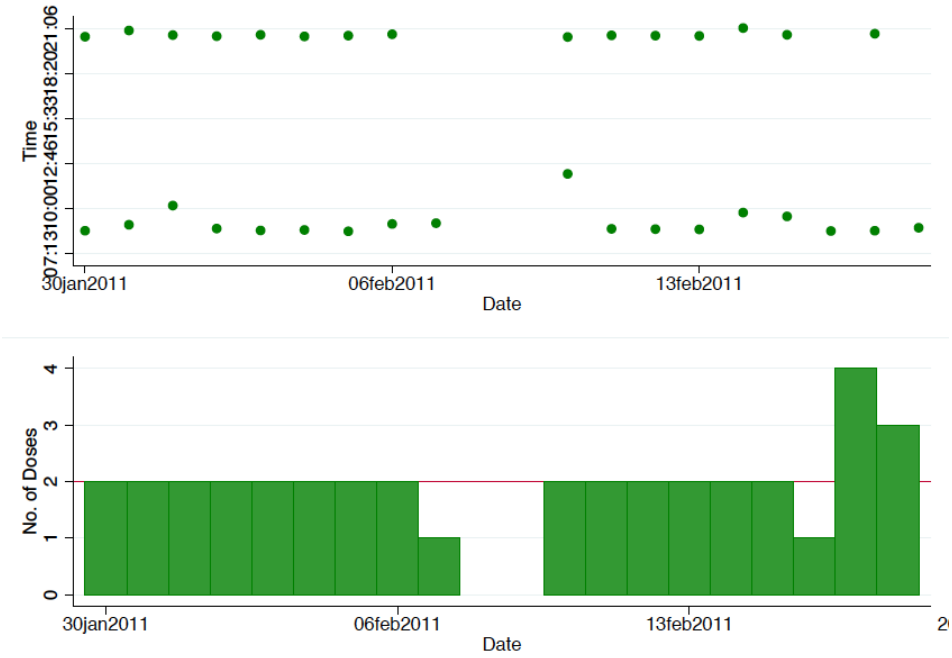

Figure 9.6: Patient 3- Scatterplot showing time of date and time of use. Green dots represent correct technique and red dots represent a technique error. Bar graph shows number of doses taken per day with a target of 2 doses per day (red line). Green bars represent correct technique and red bars represent technique errors. **This patient has good temporal and technique adherence (Class 2).**

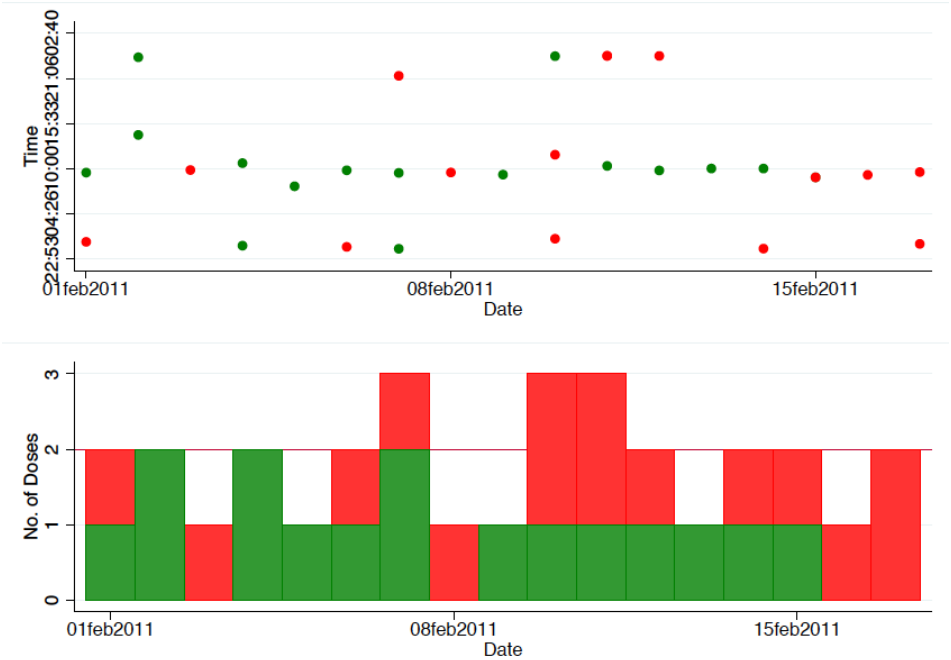

Figure 9.7: Patient 4- Scatterplot showing time of date and time of use. Green dots represent correct technique and red dots represent a technique error. Bar graph shows number of doses taken per day with a target of 2 doses per day (red line). Green bars represent correct technique and red bars represent technique errors. **This patient has poor temporal and technique adherence (Class 0).**

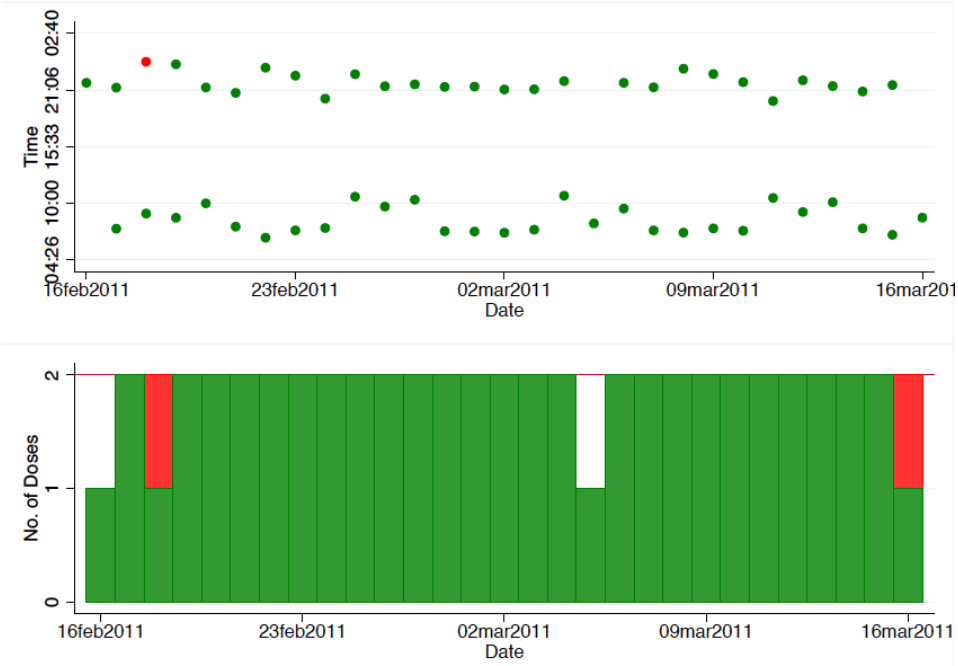

Figure 9.8: Patient 5- Scatterplot showing time of date and time of use. Green dots represent correct technique and red dots represent a technique error. Bar graph shows number of doses taken per day with a target of 2 doses per day (red line). Green bars represent correct technique and red bars represent technique errors. **This patient has good temporal and technique adherence (Class 2).**

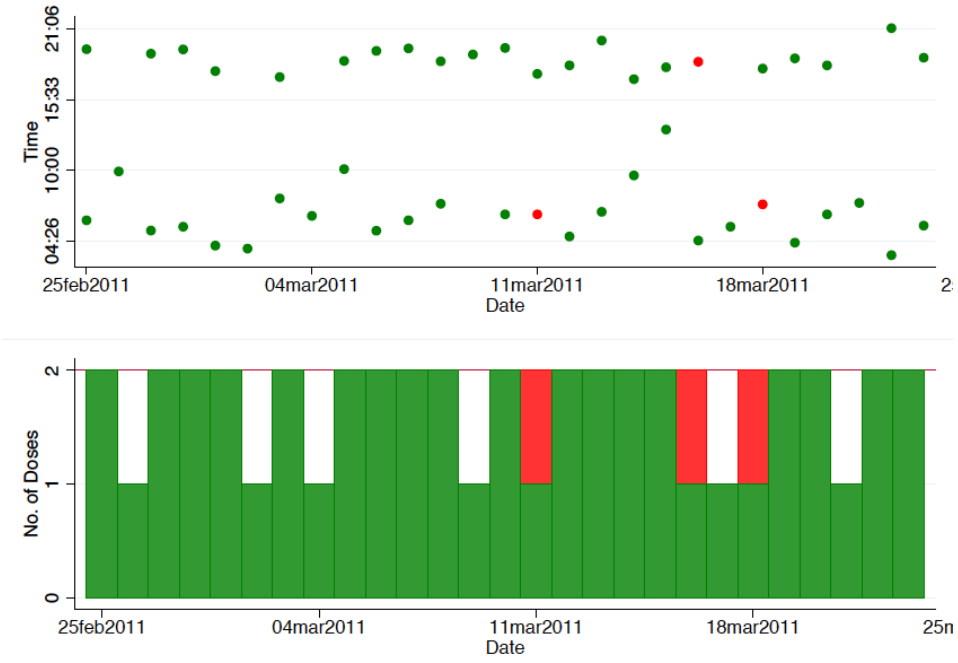

Figure 9.9: Patient 6- Scatterplot showing time of date and time of use. Green dots represent correct technique and red dots represent a technique error. Bar graph shows number of doses taken per day with a target of 2 doses per day (red line). Green bars represent correct technique and red bars represent technique errors. **This patient has relatively good temporal and good technique adherence (Class 1).**

Figure 9.10: Patient 7- Scatterplot showing time of date and time of use. Green dots represent correct technique and red dots represent a technique error. Bar graph shows number of doses taken per day with a target of 2 doses per day (red line). Green bars represent correct technique and red bars represent technique errors. **This patient has regular missed doses and technique errors (Class 0).**

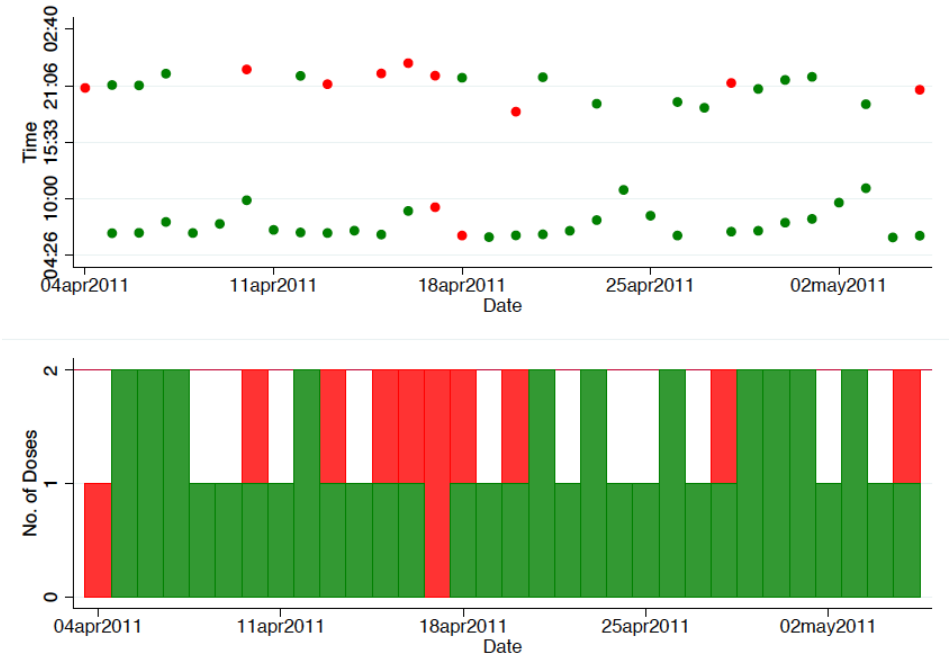

Figure 9.11: Patient 8- Scatterplot showing time of date and time of use. Green dots represent correct technique and red dots represent a technique error. Bar graph shows number of doses taken per day with a target of 2 doses per day (red line). Green bars represent correct technique and red bars represent technique errors. **This patient has regular missed doses and a cluster of doses with technique errors (Class 0).**

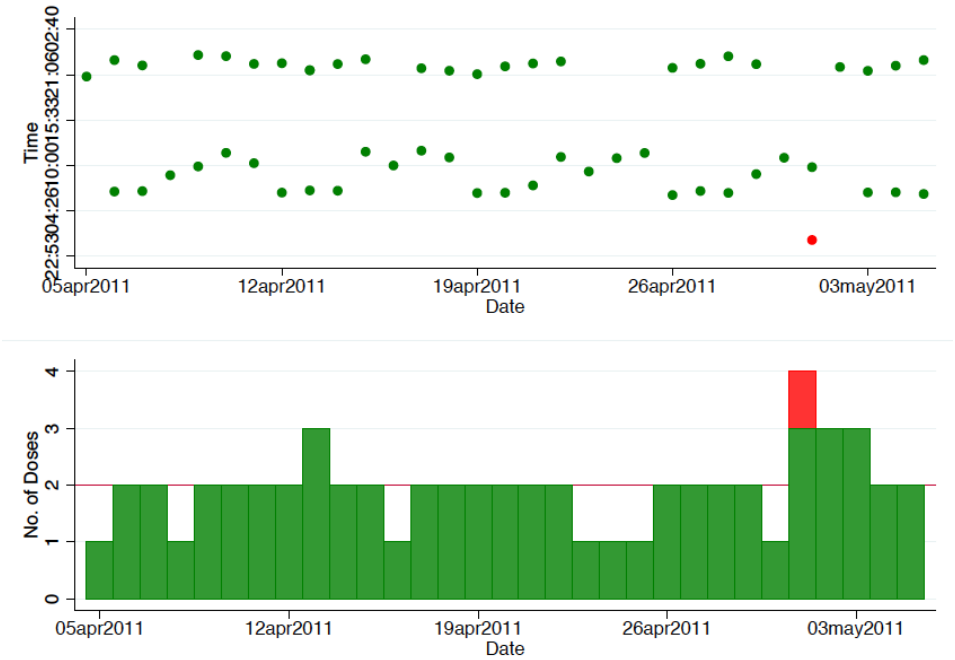

Figure 9.12: Patient 9- Scatterplot showing time of date and time of use. Green dots represent correct technique and red dots represent a technique error. Bar graph shows number of doses taken per day with a target of 2 doses per day (red line). Green bars represent correct technique and red bars represent technique errors. **This patient has moderate temporal and good technique adherence (Class 1).**

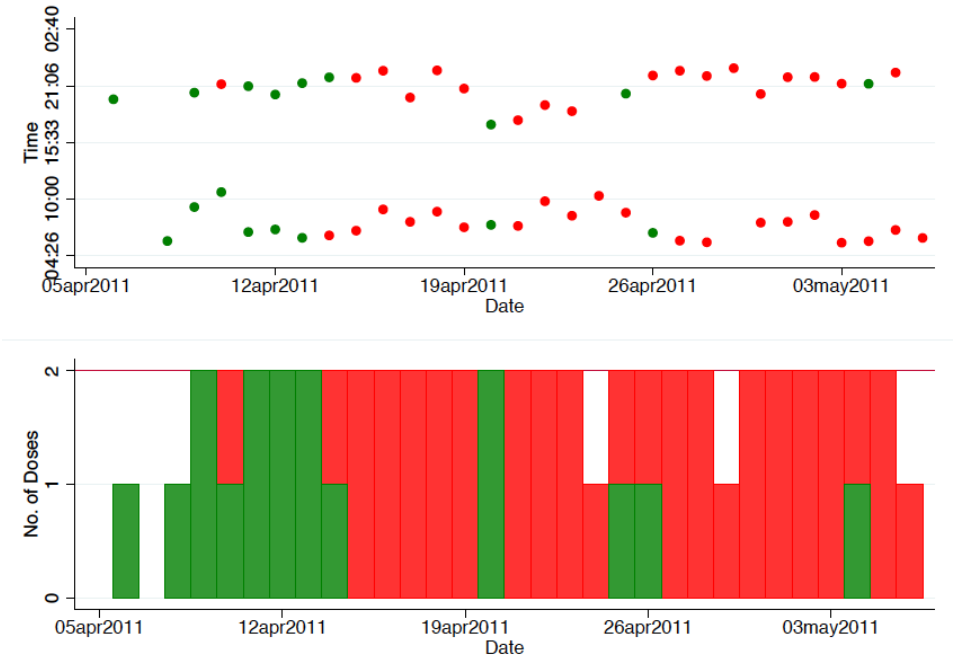

Figure 9.13: Patient 10- Scatterplot showing time of date and time of use. Green dots represent correct technique and red dots represent a technique error. Bar graph shows number of doses taken per day with a target of 2 doses per day (red line). Green bars represent correct technique and red bars represent technique errors. **This patient has poor technique adherence and good temporal adherence with only a few missed doses (Class 0).**

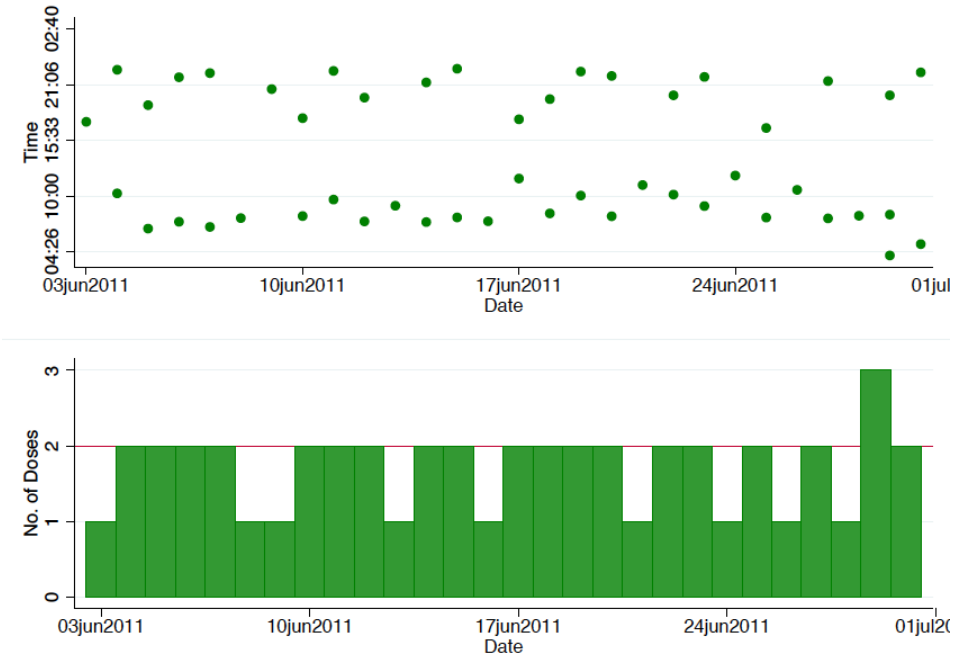

Figure 9.14: Patient 11- Scatterplot showing time of date and time of use. Green dots represent correct technique and red dots represent a technique error. Bar graph shows number of doses taken per day with a target of 2 doses per day (red line). Green bars represent correct technique and red bars represent technique errors. **This patient has regular missed doses but good technique (Class 1).**

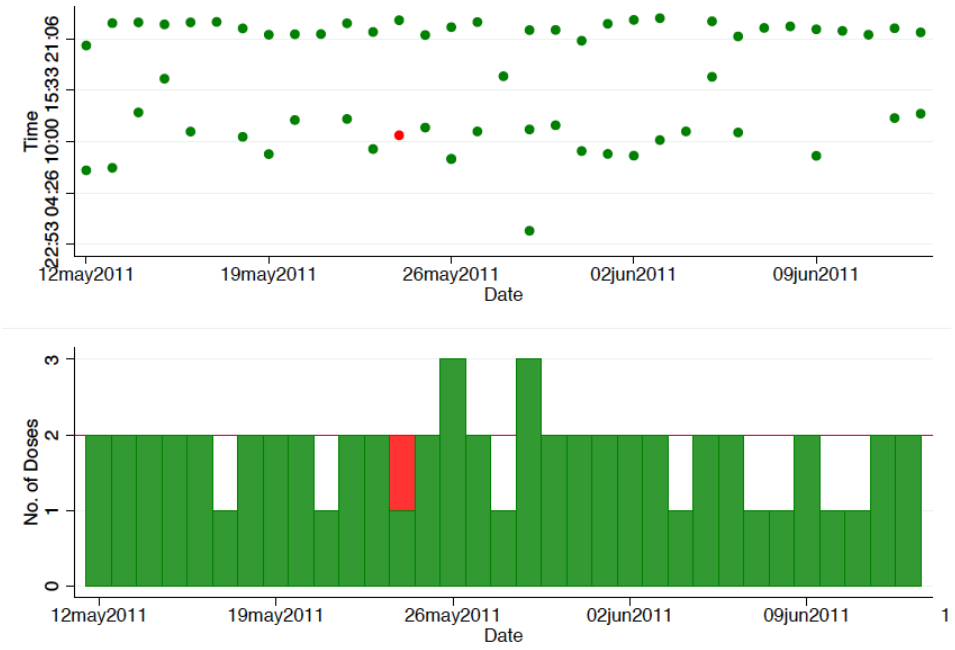

Figure 9.15: Patient 12- Scatterplot showing time of date and time of use. Green dots represent correct technique and red dots represent a technique error. Bar graph shows number of doses taken per day with a target of 2 doses per day (red line). Green bars represent correct technique and red bars represent technique errors. **This patient has relatively good temporal and technique adherence with a few missed doses (Class 1).**

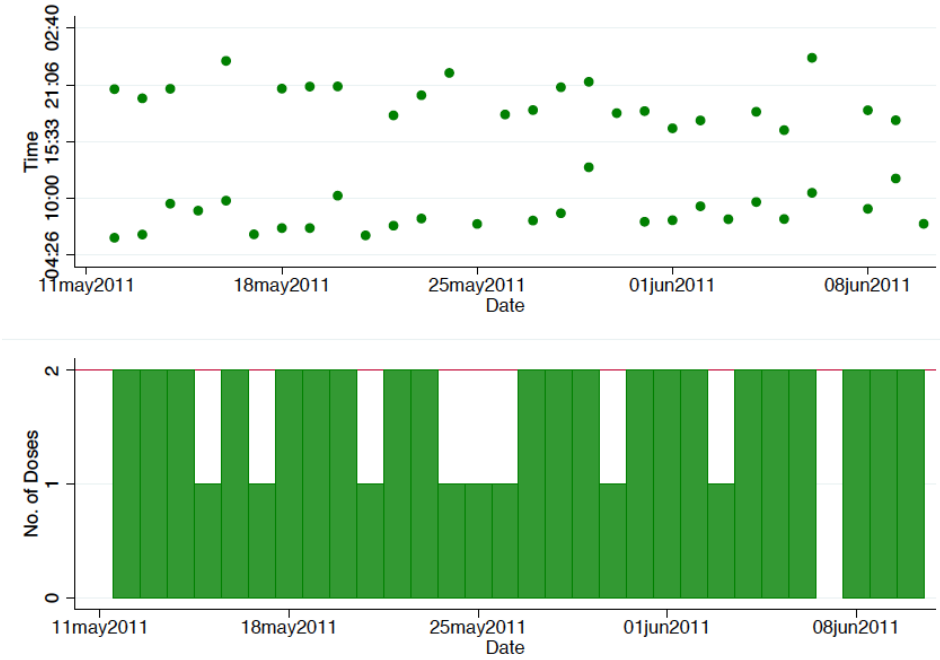

Figure 9.16: Patient 13- Scatterplot showing time of date and time of use. Green dots represent correct technique and red dots represent a technique error. Bar graph shows number of doses taken per day with a target of 2 doses per day (red line). Green bars represent correct technique and red bars represent technique errors. **This patient has moderate temporal adherence with regular missed doses and good technique adherence (Class 1).**

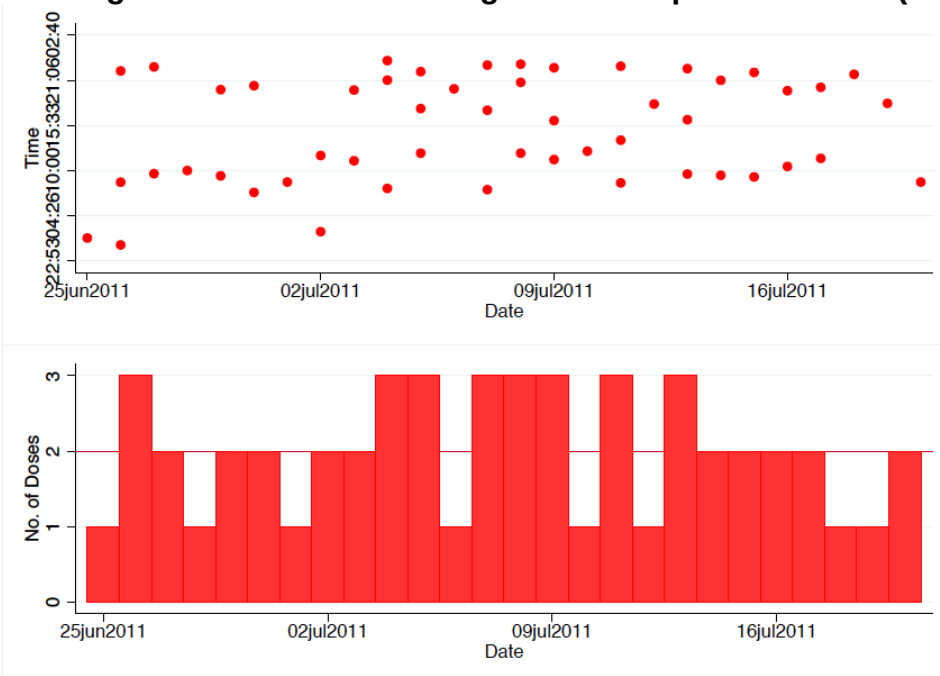

Figure 9.17: Patient 14- Scatterplot showing time of date and time of use. Green dots represent correct technique and red dots represent a technique error. Bar graph shows number of doses taken per day with a target of 2 doses per day (red line). Green bars represent correct technique and red bars represent technique errors. **This patient has poor temporal and technique adherence and both overdosing and underdosing. All events have technique errors (Class 0).**

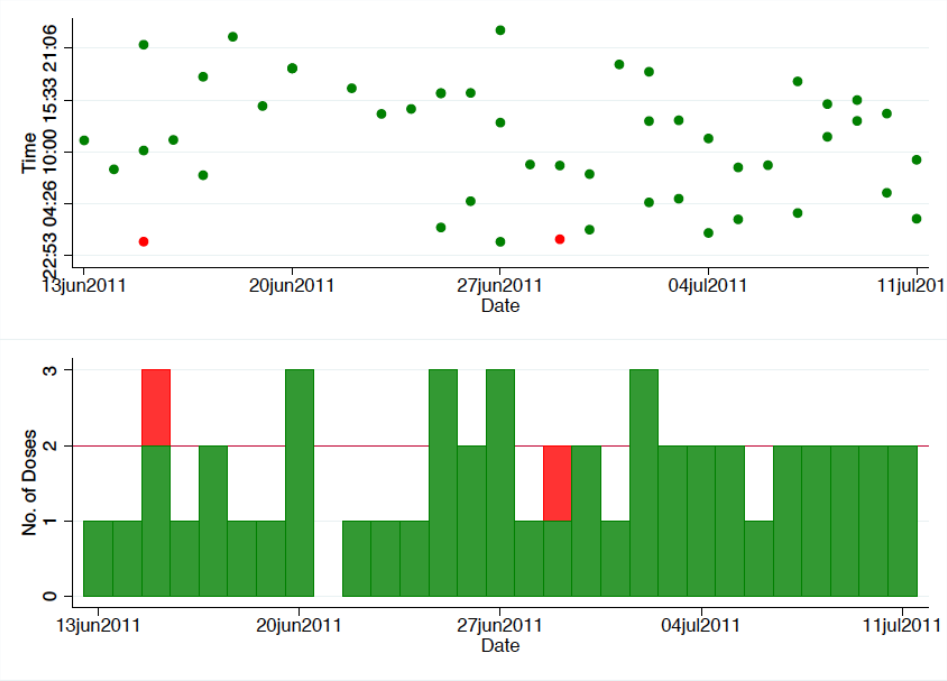

Figure 9.18: Patient 15- Scatterplot showing time of date and time of use. Green dots represent correct technique and red dots represent a technique error. Bar graph shows number of doses taken per day with a target of 2 doses per day (red line). Green bars represent correct technique and red bars represent technique errors. **This patient has poor temporal adherence with both overdosing and underdosing, as well as sporadic dose timing (Class 0).**

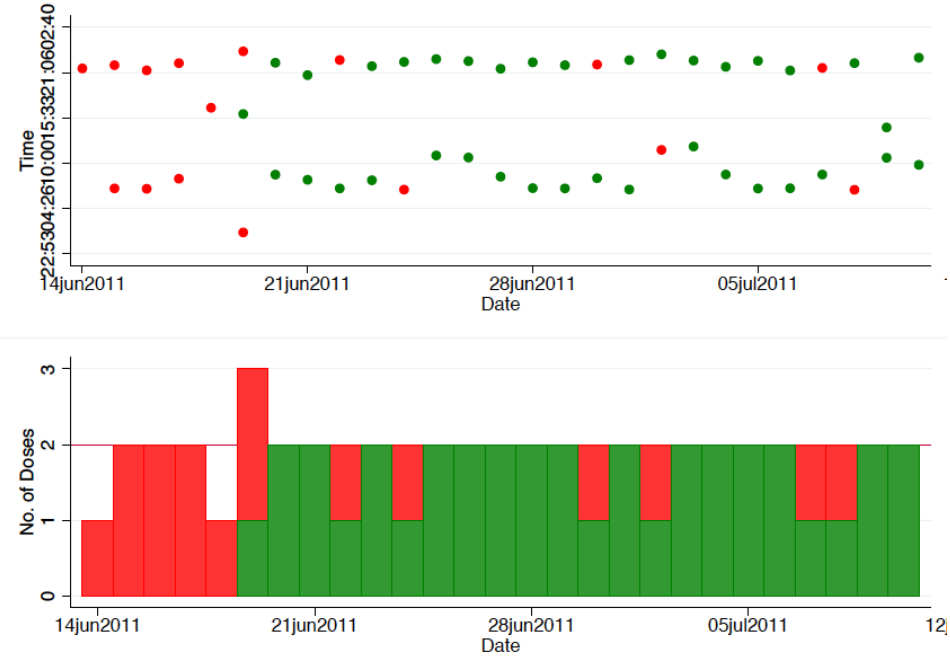

Figure 9.19: Patient 16- Scatterplot showing time of date and time of use. Green dots represent correct technique and red dots represent a technique error. Bar graph shows number of doses taken per day with a target of 2 doses per day (red line). Green bars represent correct technique and red bars represent technique errors. **This patient has good temporal adherence but poor technique adherence with a cluster of technique errors (Class 1).**

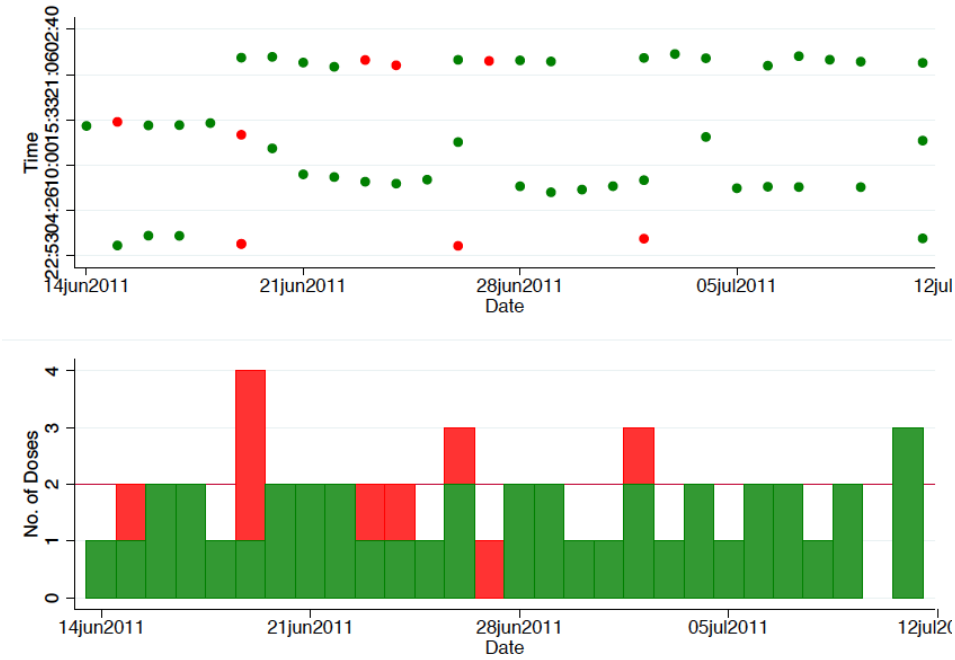

Figure 9.20: Patient 17- Scatterplot showing time of date and time of use. Green dots represent correct technique and red dots represent a technique error. Bar graph shows number of doses taken per day with a target of 2 doses per day (red line). Green bars represent correct technique and red bars represent technique errors. **This patient has moderate temporal and moderate technique adherence (Class 1).**

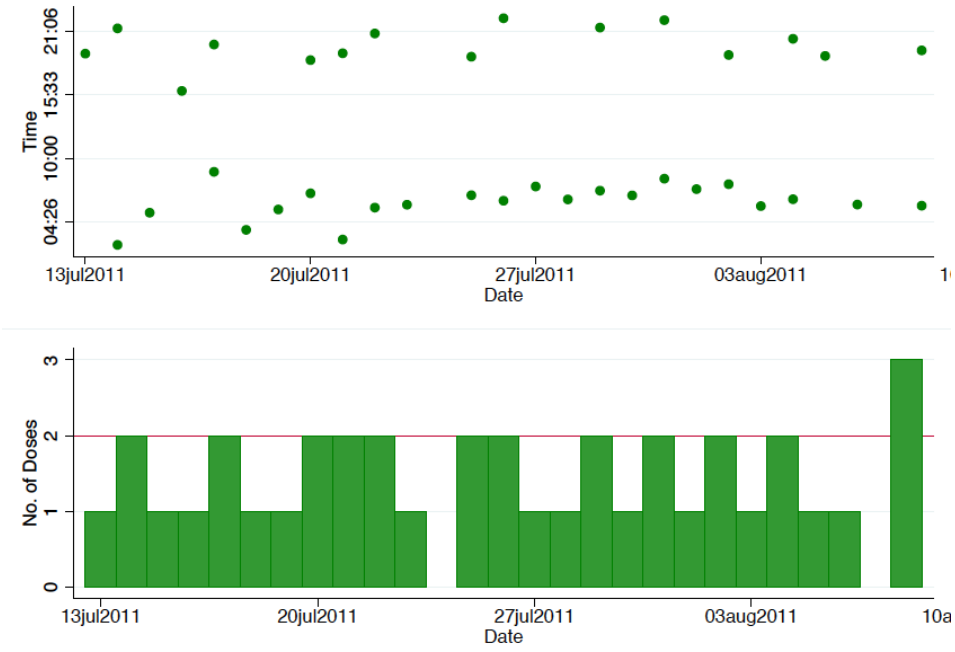

Figure 9.21: Patient 18- Scatterplot showing time of date and time of use. Green dots represent correct technique and red dots represent a technique error. Bar graph shows number of doses taken per day with a target of 2 doses per day (red line). Green bars represent correct technique and red bars represent technique errors. **This patient has poor temporal adherence with mainly missed doses (Class 0).**

Figure 9.22: Patient 19- Scatterplot showing time of date and time of use. Green dots represent correct technique and red dots represent a technique error. Bar graph shows number of doses taken per day with a target of 2 doses per day (red line). Green bars represent correct technique and red bars represent technique errors. **This patient has good temporal adherence but almost all events have technique errors (Class 0).**

Figure 9.23: Patient 20- Scatterplot showing time of date and time of use. Green dots represent correct technique and red dots represent a technique error. Bar graph shows number of doses taken per day with a target of 2 doses per day (red line). Green bars represent correct technique and red bars represent technique errors. **This patient has poor temporal and technique adherence with mainly missed doses and almost all events having technique errors (Class 0).**

A summary of the patient overall adherence classification and different adherence rates are presented in Table 9.5. The actual rate was significantly lower that all other rates (Figure 9.24) but showed the strongest correlation with overall technique classification by the raters. Based on the dose counter rate or INCA^{TM} dose rate alone, all but two patients (patients 18 and 20) were classified as good adherence (≥80%). These patients had numerous missed doses during the month of monitoring. The attempted rate would have classified both of these patients as poor adherence, as well as two others (patients 8 and 13). These additional patients also had a relatively large number of missed doses.

There was disagreement between the dose counter rate and the INCA^{TM} dose or attempted rate for patients 2, 3 and 4. These patients had episodes of multiple doses taken at the same time or dose dumping (patient 2) prior to returning the inhaler.

Table 9.5: Summary of overall rater classification, dose counter rate from Diskus^{TM} inhaler and three adherence rates calculated from INCA^{TM} device.

Subject ID	Combined temporal/ technique class	Dose counter rate	INCA^{TM} dose rate	Attempted rate	Actual rate
1	**1**	**88.2**	**92.1**	**80.3**	**59.2**
2	**1**	**178.6**	**82.1**	**87.3**	**58.7**
3	**2**	**137.5**	**92.5**	**88.5**	**71.3**
4	**0**	**135.3**	**91.2**	**89.7**	**36.2**
5	**2**	**94.8**	**96.6**	**96.8**	**73.8**
6	**1**	**88.9**	**88.9**	**86.5**	**51.7**
7	**0**	**81.3**	**82.8**	**85.3**	**47.0**
8	**0**	**81.3**	**81.3**	**79.1**	**39.3**
9	**1**	**90.3**	**96.8**	**90.9**	**54.1**
10	**0**	**93.8**	**85.9**	**92.8**	**23.5**
11	**1**	**92.9**	**85.7**	**84.6**	**54.8**
12	**1**	**90.9**	**90.9**	**91.3**	**50.8**
13	**1**	**80.6**	**80.6**	**79.8**	**52.4**
14	**0**	**103.8**	**100.0**	**107.9**	**51.9**
15	**0**	**84.5**	**86.2**	**92.8**	**54.4**
16	**1**	**98.1**	**98.1**	**99.1**	**51.4**
17	**1**	**89.3**	**89.3**	**89.6**	**51.6**
18	**0**	**70.4**	**70.4**	**71.6**	**47.0**
19	**0**	**89.3**	**89.3**	**91.1**	**46.9**
20	**0**	**60.7**	**60.7**	**56.8**	**27.9**

Class 0 – poor overall adherence, class 1 – moderate overall adherence, class 2 – good overall adherence.

Figure 9.24 shows relatively good agreement between the three temporal adherence rates and highlights the outliers mentioned before with dose counter rates. All three temporal rates were poor at classifying overall adherence. There was good separation of the actual rate values according to overall classification.

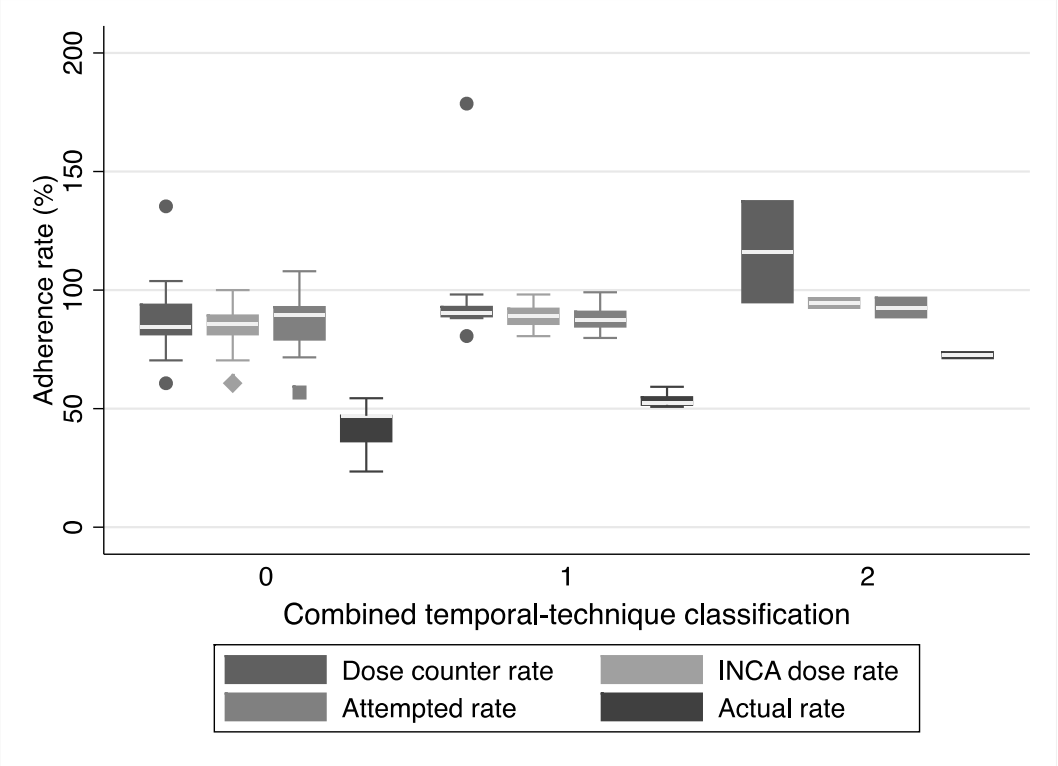

Figure 9.24: Boxplots of dose counter rate from Diskus™ inhaler and three adherence rates calculated from INCA™ device grouped by overall rater classification of adherence (n=20).

The scatterplot matrix in Figure 9.25 also highlights the lack of correlation between the three temporal adherence rates and the actual rate, thus confirming the lack of relationship between temporal dosing errors and technique errors. For example, patient 18 had poor temporal adherence and good technique adherence, patient 19 had good temporal adherence and poor technique adherence and patient 20 had poor temporal and technique adherence. The dose counter rate would have classified patients 18 and 20 as having poor adherence and would have missed the significant technique errors in patient 19's profile.

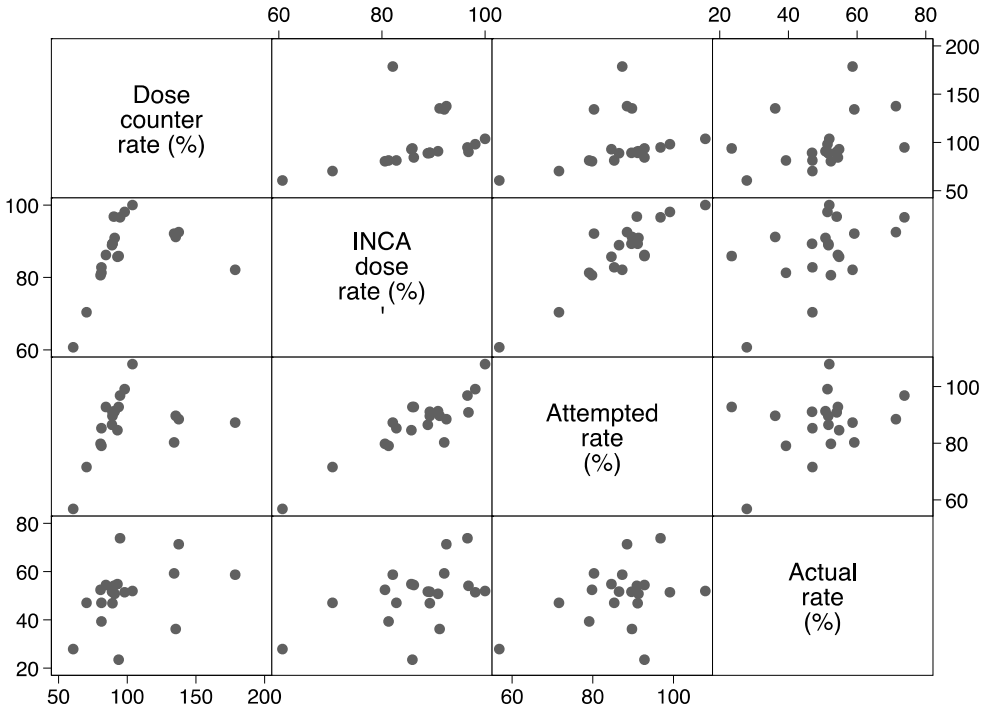

Figure 9.25: Scatterplot matrix comparing dose counter rate from Diskus™ inhaler and three adherence rates calculated from INCA™ device.

Spearman's rho and Pearson's correlation coefficients for two-way comparisons of the four adherence rates (Tables 9.6 and 9.7) demonstrate that the dose counter rate correlates poorly with the INCA™ device derived rates, most likely because of the significant outliers due to dose dumping and multiple dosing. The INCA™ rate and attempted rate were moderately correlated. The actual rate correlated poorly with all temporal rates.

Table 9.6: Spearman's rho for two-way comparisons of different adherence rates.

Rate (%)	Dose counter	INCA	Attempted	Actual
Dose counter	1.0000			
INCA	0.6601 (0.0015)	1.0000		
Attempted	0.5028 (0.0238)	0.7524 (0.0001)	1.0000	
Actual	0.4392 (0.0527)	0.3755 (0.1028)	0.1347 (0.5713)	1.0000

Table 9.7: Pearson's correlation coefficients for two-way comparisons of different adherence rates.

Rate (%)	Dose counter	INCA	Attempted	Actual
Dose counter	1.0000			
INCA	0.3645 (0.1141)	1.0000		
Attempted	0.3006 (0.1978)	0.8980 (0.0000)	1.0000	
Actual	0.3757 (0.1026)	0.5031 (0.0238)	0.3622 (0.1165)	1.0000

9.2. Discussion

This chapter sought to unify the previous experimental data and the data derived from the INCA^{TM} device into a comprehensive measure of adherence that incorporates both date and time of inhaler use and the presence or absence of technique errors and the significance of these errors. Technique errors were weighted according the experimental data presented in prior chapters.

A first-order elimination profile was used to generate trough dose levels. The mean overall trough over the study period was compared to the expected trough level if the inhaler was used as instructed to determine the attempted and actual rates (actual rates also incorporated technique errors). The benefit of a pharmacokinetic model is that it also takes into account the concept of interval adherence or the gap between doses. Even though an inhaler is used twice a day, the time of use affects the trough doses achieved. The interval adherence is not considered in the dose counter rate since data on the date and time of use is not captured. This is clearly evident for the three patients who had significant dose dumping; the dosing profiles from the INCA^{TM} device showed a marked discrepancy with the dose counter rate. In an effort to not disappoint clinicians, some patients engage in this activity of dose dumping prior to their visit to show that they have used their inhaler. Failure to capture information about actual day-to-day use would therefore be detrimental to the patient's disease control.

As expected, there was a poor relationship between the actual adherence and the temporal adherence rates. Interestingly, most patients had good temporal adherence but poor actual adherence due to the high incidence of technique errors. No patient achieved an actual rate greater than 80%, highlighting that these cutoffs may need to be adjusted to mirror real-world inhaler use where both temporal and technique errors are made.

Clinical trials on inhaler efficacy can significantly benefit from electronic monitors like the INCA^{TM} device. Results can be interpreted in the context of

actual inhaler use instead of averaged surrogate measures. Ease of use can also be determined based on the number of technique errors made. In the clinical care setting, use of the INCA^{TM} device can generate more personalized feedback to address temporal adherence, technique adherence or both. However, while this technology and method of calculating combined adherence rates seems promising, it cannot be recommended at present without further outcome studies, which can look at the clinical significance or providing feedback and training based on the dose counter and checklist (standard of care) versus the INCA^{TM} device. These trials are currently under way for various patient cohorts. (166) Larger sample sizes are also required to validate the algorithm developed in this study.

The results of this study highlight the need for a device that can monitor inhaler adherence, both temporal and technique, longitudinally since there is significant variation between patients and within a patient over time.

CHAPTER 10: General Discussion

10.1. Overview

Inhaled medications are the mainstay of therapy in the treatment of chronic respiratory diseases like asthma and COPD because they allow delivery of the active ingredient directly to the site of action. There has been a steady increase in the use of dry powder inhalers (DPIs) because they do not use chlorofluorocarbon-containing propellants of most of the older metered-dose inhalers and they have fewer coordination problems compared to metered dose inhalers (MDIs). In fact, the global market for nebulizers, inhalers and respirators was worth $12.4 billion in 2014 and is expected to grow at an annual rate of 8.9% to reach $19.0 billion by 2019.(167)

However, DPI devices are not without their problems. Good adherence to inhaled medications includes several dimensions: intensity and timing of use according to prescription (temporal adherence), continuous use (persistence) and correct use (technique adherence).(82, 168)

Problems with temporal adherence are common among all inhaler types and also other medications used to treat chronic diseases. Patient self-reporting questionnaires have shown that adherence to inhaled therapy in patients with COPD is less than 60% and the most common reason reported for poor adherence was the dosing frequency required.(169) Studies evaluating prescription filling have found that up to 20% of patients with asthma prescribed controller medications for the first time did not fill their prescriptions and the mean proportion of days covered varied from 19% to 30% over a 12 month period.(98) A similar study in Northern Ireland found that one third of patients filled fewer than half of their inhaler prescriptions and 88% admitted to poor adherence.(170) Patients with COPD generally display poorer adherence rates compared to asthmatics with up to a 60% non-adherence rate.(171-173) Poor adherence to inhaled controller medications has been estimated to account for up to 60% of asthma-related hospitalizations and increased rates of 30- and 60- day hospital readmissions in patients with COPD.(174, 175)

There are three recognized types of temporal non-adherence: underuse, overuse and haphazard use. Underuse is by far the most common type. One study found that only 15% of patients took their prescribed inhaler more than 80% of the days; on the other hand, overdosing occurred on less than 10% of days.(173)

Numerous electronic monitoring devices have been developed over the last four decades to monitor temporal non-adherence; however, many of these devices do not monitor all or most aspects of inhaler technique. Inhaler technique or competence is a very important component of overall adherence. Badder et al found that 85% of patients with poor inhaler technique demonstrated poor asthma control.(176) Many features specific to inhaler design contribute to technique adherence; errors are more common with the Turbuhaler™ (32%) and pMDI (28%) compared to the Diskus™, Autohaler and Aerolizer (11-12%).(177) In some studies, up to 90% of patients make at least one inhaler technique error.(178) The most common error types are also device-specific: low breath-hold duration and exhalation prior to inhalation are most common with the Diskus™, Turbuhaler™ and Aerolizer, whereas errors with inhalation are most common with the Autohaler or pMDI.(177)

Currently used methods for monitoring inhaler technique are suboptimal. Checklists are very subjective and other methods such as the Clement-Clarke In-Check Dial™ assess only one or a few components of technique, such as inhalation flow rate.(61, 62, 132, 133) Most methods are a once-off assessment in the clinic setting unless the clinician relies on the patient to document readings at home, in a similar fashion to peak expiratory flow rate readings. The data on the effect of certain errors on drug delivery and efficacy is also lacking. The strongest level of evidence exists for the effect of errors in inhalation or breath hold duration, although most of the studies have used in vitro or in silico methodologies.(75, 85, 86, 89, 94, 96)

There was a need to study the frequency of temporal and technique non-adherence in the Irish population and to investigate the impact of dosing and

technique errors on drug delivery. Moreover, a comprehensive system of tracking the date and time of inhaler use, as well as, the presence or absence of technique errors on a daily basis was essential to not only an epidemiological understanding of inhaler use but to tailoring of inhaler training and clinical care plans to individual patients.

A collaborative effort by physicians and engineers has allowed the development of an acoustic monitor, the INCA^{TM} device, which can be attached to the Diskus^{TM} inhaler, in order to monitor both how and when the inhaler is used. While the accuracy of the device for monitoring date and time of use has been studied by the group, the ability to accurately assess inhaler technique and the correlation between these errors and drug delivery have not.

This thesis aimed to describe features of temporal and technique adherence to a common DPI in a community care Irish population and to study the effects of Diskus^{TM} inhaler technique errors on drug delivery. These observations were utilized to develop and validate an algorithm to incorporate data on temporal and technique adherence obtained from the INCA^{TM} device into a single overall adherence metric.

10.2. Main findings

10.2.1. How common are Diskus™ inhaler temporal and technique errors in a community care setting?

The dose counter over-estimated the number of doses taken (55 per patient versus 49 per patient with INCA™ device). Only 20% of patients took their inhaler in the correct manner at the correct interval and missed doses were more common than extra doses. Three-quarters of the acoustic profiles demonstrated good inhaler technique. The most common inhaler technique errors identified were poor peak inspiratory flow rate (27% of inhalations), multiple inhalations (25%), dose blistering without an inhalation (19%) and exhalation into the inhaler after blistering but before inhalation (18%). The mean number of errors per person was 12 per 60-dose inhaler. Overall adherence changed during the month of observation in two groups of patients; adherence improved in one group and worsened in another.

10.2.2. The effect of inhalation parameters on delivered dose and an acoustic method to quantify this effect

The mean Diskus™ PIFR was lower in patients with COPD and Neuromuscular disease compared to asthmatics and healthy individuals. Diskus™ PIFR was also lower in older patients (>50 years). There was a moderate correlation between Diskus™ PIFR and spirometric PIFR. Spirometric PIFR may be used as a surrogate measure of PIFR through the Diskus™ inhaler.

In a study of healthy volunteers, there was a strong correlation between the amplitude of inhalation, in particular the mean absolute deviation of amplitude and the average power in the 300-600 Hz band, with the PIFR generated through the Diskus™ inhaler. Volume of inhalation through the Diskus™ could be calculated using the Diskus™ PIFR and duration of inhalation.

In a respiratory disease cohort, MAD amplitude had the strongest correlation with Diskus™ PIFR (r=0.884). The equations for calculating Diskus™ PIFR generated from data collected from healthy individuals demonstrated greater than 90% sensitivity and specificity for classifying an inhalation (correctly classified 95% of inhalations > 30 l/min, 91% > 45 l/min, 93% > 60 l/min and 92% > 90 l/min). Estimated Diskus™ PIFR and duration of inhalation accounted for greater than 95% of the variation observed in fine particle fraction emitted from the salmeterol/ fluticasone inhaler and Diskus™ PIFR accounted for more than 70% of the variation observed in FPF from the salbutamol inhaler. The FPF from the salbutamol inhaler was more than double the FPF from the salmeterol/ fluticasone inhaler and the mean mass aerodynamic diameter (MMAD) was lower for the salbutamol inhaler.

Pharmacokinetic studies on 10 healthy volunteers demonstrated that there was a significant two-fold increase in peak salbutamol concentration (measured at 20 min post-dose) achieved when Diskus™ PIFR was above 60 l/min compared to when Diskus™ PIFR was below 60 l/min for each individual. Diskus™ PIFR explained more than 50% of the variation observed in peak salbutamol concentration.

10.2.3. Development and validation of an acoustic method to detect and quantify the effect of exhalation into a Dry Powder Inhaler

Exhalation directed at the inhaler mouthpiece after dose blistering was found to have an overall negative effect on drug delivery. At an exhalation distance of 0 cm from the inhaler mouthpiece, less than 50% of drug available is delivered on average for all flow rates using humid air. In a worst-case scenario, an average of 2.44% of drug is delivered after an expiratory flow rate of 120 L/Min, at a distance of 0 cm from the inhaler mouthpiece. The fine particle fraction (FPF) was significantly reduced for inhaler devices subjected to an exhalation.

A strong correlation was observed between the exhalation flow rate, the distance of the exhalation from the mouthpiece and the direction of the

exhalation with the acoustic features obtained from the exhalation audio signal. An algorithm was developed to classify an exhalation as significant (exhalations occurring at a distance of 5 cm or less, into the DPI mouthpiece or directly at the INCA™ device). The algorithm had a sensitivity of 72.22% and a specificity of 85.71%.

10.2.4. Does orientation of the Diskus™ inhaler affect available dose for delivery?

Only when the Diskus™ was held at the 90° position after dose actuation and shaken was significant amount of drug removed (54% of available dose removed with a range of 39.4% to 62.3%). Tapping the Diskus™ at a position of 90° removed approximately 10% of the available dose. Holding the Diskus™ in a steady position at 45° or 90° had no appreciable effect on the amount of drug available for delivery.

10.2.5. Impact of breath holding on drug delivery

There was a statistically significant 29% reduction in trough salbutamol concentration when subjects had only a 4 second breath hold duration compared to correct inhaler technique with a 10 second breath hold. There was also a 22% lower rise in salbutamol concentration from the dose 6 trough level when using a 4 second breath hold.

10.2.6. Impact of missed doses on steady state trough and peak levels

A theoretical model based on first-order kinetics and a salmeterol/ fluticasone functional half-life of 12 hours was used to estimate the impact of missing doses 3 and 4 in a 6-dose regimen. The expected mean pre-dose 6 trough level when doses 3 and 4 was 36% lower than when all 6 doses are taken. Pharacokinetic studies on 7 healthy volunteers demonstrated a statistically significant 64% difference in pre-dose 6 trough concentration between the control group and the group that missed doses 3 and 4 (control: 0.85 ng/ml and missed doses: 0.55ng/ml, $p<0.001$).

10.2.7. Development and validation of an algorithm for combining time and technique of inhaler use into a single metric

A pharmacokinetic algorithm was developed to combine date and time of inhaler use with the presence or absence of technique errors into a single measure called the actual rate. The algorithm was tested in a cohort of twenty patients followed for one month with the INCA^{TM} device. There was significant variation in both temporal and technique adherence. Some patients had very erratic dosing with both over dosing and under dosing, others had predominantly under dosing and others had significant numbers of technique errors (three patients had all or almost all doses with technique errors). The actual rate was significantly lower that all other rates but showed the strongest correlation with overall technique classification by the raters. The dose counter rate correlated poorly with the INCA^{TM} device derived rates, most likely because of the significant outliers due to dose dumping and multiple dosing, as well as the failure to detect technique errors.

10.3. Contextual discussion

This body of work highlights the utility of the INCA™ device in not only logging and monitoring the date and time when a Diskus™ inhaler is used but also in detecting the presence or absence of critical technique errors, which may be detrimental to drug delivery and efficacy. The Diskus™ is one of the two most commonly prescribed DPIs in developed countries.(65) The INCA™ device was designed to be attached to the Diskus™ and to monitor inhaler use over a one-month period.

Chapter 3 showed that temporal and technique adherence was relatively poor in an Irish community care setting, with only 20% of patients using their Diskus™ DPI as prescribed. The most common technique errors identified were poor peak inspiratory flow rate, multiple inhalations, exhalation prior to inhalation and dose blistering without a subsequent inhalation.

Studies normally highlight exhalation errors as the most common Diskus™ technique error.(81) The rate of inhalation errors is comparatively low in the literature.(177) The reason for this discrepancy is likely the lack of an objective universal method of assessing peak inspiratory flow rate and the variability in flow rate from day to day. Although the Clement-Clarke In-Check Dial™ and the Vitalograph AIM are available to estimate the PIFR achieved from various devices, these devices are not universally used by clinicians in evaluating inhaler technique and thus, assessment of inhalation is quite subjective. The mouthpiece dimensions of the In-Check Dial and AIM are also quite different and may have an effect on the mouthseal achieved by the patient or the general way the patient handles the peak flow meter versus the Diskus™ inhaler.

The evidence presented in Chapter 4 highlights that peak inspiratory flow rate generated through the Diskus™ inhaler has a significant effect on drug delivery both in vitro and in vivo, mirroring the evidence in the literature. The INCA™ device had excellent sensitivity and specificity for classifying an inhalation as adequate (>60 l/min), suboptimal (30-60 l/min) or inadequate

(<30 l/min). The advantage of the INCA^{TM} device compared to other peak flow meters is that it can be used to monitor PIFR longitudinally. We have seen that patients in a community care setting do not necessarily make the same inhaler error on every dose; likewise, there is significant variability in PIFRs generated within an individual over time. These findings may be related to acute exacerbations or other psycho-social factors, such as the patient's assessment of disease control. It is therefore, more beneficial to look at patterns of inhaler use rather than a once-off evaluation of inhaler technique.

"Multiple inhalations" was the second most frequent error type. Many patients with asthma are either current or former users of spacer devices with pMDIs. Spacers allow increased drug delivery by decoupling the need to coordinate dose actuation with inhalation.(179) The user takes multiple deep breaths in and out through the device. Many patients have either used this "multiple inhalation" technique or seen it being used and this may explain why one-quarter of patients demonstrated a similar technique when using the Diskus^{TM} inhaler. Unfortunately, "multiple inhalations" with a DPI usually shortens the breath hold duration. Chapter 7 highlights that a shorter breath hold duration leads to reduced drug delivery and this effect is similarly seen in numerous in silico models of breath holding in the literature.(75, 86) Coughing immediately after inhalation is also a significant problem. ICSs can lead to bronchospasm and cough after inhalation and cough is a recognized feature of COPD and asthma.(180) Coughing also shortens the breath hold duration, thus impacting drug delivery. The INCA^{TM} device is able to detect both multiple inhalations and episodes of coughing after inhalation and can accurately quantify the breath hold duration if these events occur.

Exhalation into the inhaler mouthpiece after blistering but before inhalation is another common technique error in our patient cohort. In vivo studies in Chapter 5 showed that exhalation of humidified air directed towards the Diskus^{TM} mouthpiece had a significant effect on the amount of drug available for delivery and the subsequent fine particle fraction when inhaled. While studies on Diskus^{TM} technique have highlighted that this error is very common, there is no evidence in the literature quantifying the effect of exhalation on

drug delivery. The mechanism underlying the finding of reduced FPF is most likely clumping of the active drug and carrier inside the dose blister, limiting the subsequent de-aggregation usually observed with inhalation.

Diskus™ orientation or position during use is frequently assessed in checklists of inhaler technique.(76, 181) The rationale for inclusion of this error is the fact that after dose blistering, the drug is now exposed and can be displaced from the inhaler if not held in a horizontal or upright position. The effect of Diskus™ position has either never been studied formally or has been studied by the manufacturer but not published. The results of Chapter 6 show that Diskus™ position has a minimal impact on the amount of drug removed from the inhaler unless the inhaler is held with the mouthpiece facing down and the inhaler is tapped or shaken. While the INCA™ device cannot detect Diskus™ orientation in space, it can detect when the device is shaken vigorously (another common error since patients usually shake their pMDI prior to use) or tapped.

Published estimates of temporal adherence in the literature have been based on pharmacy records of filled prescriptions and on subject patient reports; these estimates are either inherently biased in the case of patient reported adherence or only give averages of adherence over a period of time.(98, 169) New electronic monitors, which log the date and time of inhaler use have allowed better estimates of patient adherence. The INCA™ device allows accurate logging of the date and time of inhaler use allowing the physician to recognize patterns of inhaler use and relate these patterns to patient-centric factors. The results presented in Chapter 8 show that missed doses can have a significant impact on drug delivery, using serum concentrations as a surrogate measure. The theoretical pharmacokinetic model shows that the number of missed doses and which dose is missed in relation to the time of trough concentration measurement can also impact measured serum concentrations. It is recognized that many patients, who are enrolled in clinical trials of inhaled drugs, demonstrate the Hawthorne effect, that is, adherence rates are good at the beginning of the trial and subsequently worsen when clinician contact is reduced. Chapter 3 shows similar findings, in that a group of patients monitored over a month demonstrated good temporal adherence

on enrollment and had a gradual decline in adherence over the month of observation. The data highlights the need to be able to track temporal adherence and patterns of temporal adherence over time.

Based on the available evidence in the literature and the results of studies reported in Chapters 3 – 8, Diskus™ inhaler errors were classified as either critical or non-critical. An algorithm was developed to predict effect small airways concentrations of active drugs based on the half-life of the drug (12 hours), the date and time the inhaler was used, and the predicted fine particle fraction delivered, which was affected by the presence of technique errors. This is the first study of its kind attempting to combine features of temporal and technique adherence in a single metric called actual adherence. The results of Chapter 9 show that there was a discrepancy between the INCA™ dose rate and the dose counter rate, especially when patients engaged in dose dumping. Dose dumping has been recognized in prior trials using electronic monitoring devices such as the Nebulizer Chronologs, where the authors concluded that deception among noncompliers occurs frequently in clinical trials, is often not revealed by the usual methods of monitoring, and cannot be predicted by data readily available in clinical trials.(182) The INCA™ device allows another layer of detection of this phenomenon. Not only can the number of dose actuations in a short period of time be detected but the INCA™ device can highlight whether these actuations were associated with subsequent inhalations (representing over-dosing) or not (representing dose dumping).

Finally, Chapter 10 highlighted again the frequency of technique errors since the actual adherence rate was significantly lower than measures of only temporal adherence derived from either the dose counter or the INCA™ device. There was also very little correlation between actual adherence and temporal adherence, meaning that patients with good temporal adherence did not necessarily make fewer technique errors. The single metric of actual adherence also has a pharmacologic basis and is likely to better predict drug efficacy and simple temporal adherence rates.

10.4. Limitations

This thesis did not report on all Diskus™ inhaler technique errors; only errors that could potentially be detected by the INCA™ device were studied, which still accounted for the large majority of Diskus™ technique errors in the literature. For example, the failure to exhale to residual lung volume prior to inhalation and its effect on subsequent fine particle fraction delivered was not studied here and has been reported in the literature to be a common technique error in Diskus™ users.(177) The effect of neck extension on fine particle fraction or upper airways dose was also not investigated. Some errors, such as failure to open the device or failure to actuate or blister a dose, were considered to lead to 0% dose availability due simply to the design of the Diskus™ and hence, did not require further study. Other errors, such as mouth rinsing after inhalation, while important from the point of view of side effects, were not important for drug delivery or efficacy and were omitted from this thesis.

One major shortcoming of the INCA™ device is its inability to detect the breath hold duration if an exhalation is not audible after the dose inhalation or if the device is closed prior to this exhalation. Some patients do make audible exhalations so that the breath hold duration can be quantified and the duration can also be quantified in the case of multiple inhalations or coughing after inhalation.

The acoustic method developed for estimating inspiratory flow rate is also not flawless and a few patients with adequate or suboptimal flow rates were misclassified into either suboptimal or inadequate categories, respectively. The correlation between Diskus™ PIFR and peak serum salbutamol concentrations was only moderate and PIFR could not explain a large proportion of the variation in peak concentrations, likely reflecting that other inhaler technique factors or biological factors, such as drug absorption, were not accounted for. This highlights a major flaw in using serum drug concentrations to predict local accumulation of the drug at the site of action (the small airways). The assumption is made that the two are correlated but

there is likely intra- and inter-individual biological variation in drug absorption. The ideal method of monitoring drug accumulation or residence time in the small airways is the use of radionucleotide tags but this is substantially less safe than blood sampling, especially with repeated doses.

Further limitations of the in vivo studies reported in this thesis are the small sample sizes and the use of healthy volunteers only. Most pharmacokinetic studies of inhaled medications have been limited to a sample size of 10 historically due to the extensive blood sampling required. Since the studies reported here utilized a repeated measures design with a washout period, the number of recruits available was rather small. The physiology and anatomy of the lungs in healthy individuals is also significantly different to that of patients with chronic airways diseases and this may affect drug delivery and serum concentrations achieved. It is therefore difficult to generalize findings in a small group of healthy volunteers to larger asthma and COPD populations.

This body of work also suffers from a lack of data on pharmacodynamics and a lack of outcome data. While the trough concentration may be correlated with clinical effect, no drug specific effects such as bronchodilation or change in FEV1 were studied. Similarly, the longitudinal studies did not investigate the relationship between the INCA^{TM} device measures of temporal and technique adherence with rates of exacerbation, use of reliever medications or overall disease control. Collection of these outcome measures would require a longer study period and a randomized controlled design.

10.5. Future research and applications

Studies are in progress to evaluate whether patient feedback based on the INCA™ device is superior to the standard of care in the treatment of asthma.(166) These studies are monitoring outcome data such as quality of life scores (Asthma Quality of Life Questionnaire), disease control scores (Asthma Control Test) and objective measures of control such as peak expiratory flow rates, exacerbation rates and use of reliever inhalers. Similar studies are being conducted in COPD cohorts.

The INCA™ device is also being adapted to the MDI inhaler, allowing accurate monitoring of reliever inhaler use in future clinical trials and patient care.(183) The aim is to extend the patented technology to other DPIs, including the Turbuhaler™ and the new ELLIPTA™. The ELLIPTA™ is Glaxo-Smith Kleine's new inhaler, which is touted to be more user-friendly than the Diskus™ and allows once daily dosing.(184) However, the device has its unique technique error profile than can affect drug delivery and would still benefit from an adherence monitor.

Further studies are necessary to advance the work conducted here into the effect of technique errors in asthma and COPD cohorts. These large trials will be able to correlate the measures of drug delivery predicted by the INCA™ algorithms with actual drug delivery, either from surrogate trough concentrations or pharmacodynamic data including FEV1, PEFR or fraction of exhaled nitric oxide (FENO). There is increasing interest in the correlation between FENO measurements, which are simple and non-invasive, and non-adherence or asthma disease control.(185)

The two main areas where the INCA™ technology can be applied are in clinical trials of new inhaled therapies, where accurate data on adherence is necessary to adjust for the effect of this major confounder, and in routine patient care, where data from the device can allow the physician or nurse practitioner to tailor educational training and feedback.

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APPENDICES

APPENDIX 1: Ethics approvals and patient information leaflet.

Website: www.beaumont.ie

Ospidéal Beaumont

BEAUMONT HOSPITAL
P. O. Box 1297 Beaumont Road Dublin 9
Telephone: 809 3000 / 837 7755 Facsimile: 837 6982

Patient Information Leaflet

(Assessing inhaled drug delivery through sound analysis)

Study title: Dosing algorithm for inhalational therapy using acoustics

Principal investigator's name: **Professor Richard W. Costello**

Telephone number of principal investigator: **00353-1-809-3762**

You are being invited to take part in a clinical research study to be carried out at Beaumont Hospital.

Before you decide whether or not you wish to take part, you should read the information provided below carefully. Take time to ask questions – don't feel rushed and don't feel under pressure to make a quick decision.

You should clearly understand the risks and benefits of taking part in this study so that you can make a decision that is right for you. This process is known as 'Informed Consent'.

You don't have to take part in this study and a decision not to take part will have no effect on your future medical care.

You can change your mind about taking part in the study any time you like. Even if the study has started, you can still opt out. You don't have to give us a reason. If you do opt out, rest assured it won't affect the quality of treatment you get in the future.

Why is this study being done?

We want to measure how well a drug gets into your system and see if we can relate that with the sounds of breathing through an inhaler.

Who is organising and funding this study?

Professor Richard Costello and his research team are organising this study. This is an investigator-led study and is being funded by the Health Research Board of Ireland. The results of this study will be used to write a thesis for one of Professor Costello's researchers with the aim of obtaining a degree.

Why am I being asked to take part?

You have been approached to consider taking part in this study because you are a healthy individual or have mild obstruction from asthma on your breathing tests.

How will the study be carried out?

The study will commence in June 2013 and recruitment will continue until April 2015 (2 years). The study will take place at Beaumont Hospital under the management of Professor Costello and his research team and with engineers in TCD. For you there is only one set of tests required and they take about 60 minutes to complete.

What will happen to me if I agree to take part?

If you take part we will ask you to have basic lung function tests done. We will also instruct you on how to use a Diskus inhaler. You will be enrolled in this study for a period of three (3) weeks. On week one, you will take a Salbutamol Diskus inhaler regularly for one week. You will have blood samples taken, lung function tests and Blood Pressure/ Heart Rate monitoring done during this period. This will be followed by a week where no drug is taken. In the final week, the above will be repeated with a change to the dosing frequency or how the inhaler is used.

What are the benefits?

There are no immediate benefits to your participation in this study.

What are the risks?

The needle into the arm can be sore and there is a small risk of infection, although by using the best sterile techniques we will keep this risk to a minimum.

The medicine, salbutamol can cause shaking or fast beating of the heart although this is unlikely when a person takes a single dose.

Is the study confidential?

Your GP will not be informed of your participation unless this is specifically requested by you, as there is no new treatment or change in therapy.

All your information will be viewed only by members of Professor Costello's team and be stored on encrypted, password protected computer systems. The sounds of your breathing tests will be analysed by engineers in Trinity College Dublin, although they will not know your name or identity. The blood samples will not be kept after they have been analysed.

All your results and your personal information from the study will be stored for a period not exceeding ten years after which they will be destroyed as per hospital protocol, i.e. shredding and deletion.

All data we collect through this research study will be 'coded'. This means that only members of Professor Costello's research team will be aware of the code and therefore your identity when looking at the data.

The results obtained from this study will be published in a scientific journal, no patient information will be published.

Where can I get further information?

If you have any further questions about the study or if you want to opt out of the study, you can rest assured it won't affect the quality of treatment you get in the future.
If you need any further information now or at any time in the future, please contact:

Name: Prof. Richard W. Costello

Address: Department of Medicine, Education & Research Centre,

Beaumont Hospital, Dublin 9
Phone No: (353) 01-8093762 (office hours only)

Beaumont Hospital
Ethics (Medical Research) Committee

21st June 2013

REC reference: 13/ 36

Prof. Richard Costello
Consultant Physician
Department of Medicine
Smurfit Building

Dear Prof. Costello

RE: 13/36 – Prof. R. Costello – Monitoring Lung Function through Sound Analysis

Please find enclosed ethics approval documentation for the above study, and both Parts thereof – Part 1A – which records breathing sounds of patients undergoing breathing tests; and Part 1B – which records breathing sounds of patients undergoing a Histamine Challenge.

Please ensure that the Patient Information Leaflet for Part 1B is footnoted V2, 4 June 2013 prior to recruitment.

With best regards

Yours sincerely

Ms. Gillian Vale
Administrator
Ethics (Medical Research) Committee

c.c.

Dr. Jansen Seheult
c/o Prof. Richard Costello
Department of Medicine
Smurfit Building

Ethics (Medical Research) Committee - Beaumont Hospital
Notification of ERC/IRB Approval

Principal Investigator: **Prof. R. Costello**

REC reference: **13/36**

Protocol Title: **Monitoring Lung Function through Sound Analysis**

Part 1A = Recording breath sounds during breathing tests

Part 1B = Recording breath sounds during a histamine challenge test

Ethics Committee Meeting Date: **12th April 2013**

Final Approval Date: **21st June 2013**

From: **Ethics (Medical Research) Committee - Beaumont Hospital, Beaumont, Dublin 9**

Documents Reviewed

Document and Date	Date Reviewed	Approved
Application, V2, 25/5/13	**21/6/13**	**Yes**
Patient Information Leaflet, (Part 1A) V2, 4/6/13	**21/6/13**	**Yes**
Patient Information Leaflet, (Part 1 B) V2, 4/6/13	**21/6/13**	**Yes**
Patient Consent Form (Part 1A & 1B) V1, 1/3/13	**21/6/13**	**Yes**
CV: R. Costello	**On File**	**On File**
Cert of Insurance, RCSI	**On File**	**On File**

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Prof. Alice Stanton
ERC/IRB Convenor's Signature
Approval # 1, dated 21st June 2013

Terms of Approval

- The protocol and research must comply with all relevant Irish legislative requirements and the researchers must abide by the ethical principles outlined in the Declaration of Helsinki and Good Clinical Practice.

- Prior approval from the Ethics Committee must be sought for any proposed changes/amendments to this protocol and research.

- Annual Progress Reports and a Final report must be supplied to the Ethics Committee.

FIRST REPORT DUE: JUNE 2014

FINAL REPORT DUE: JUNE 2015

- All relevant information about serious adverse reactions and new events likely to affect the safety of the subjects must be reported to the Ethics (Medical Research) Committee in writing.

The Irish College of General Practitioners

Coláiste Dhochtúirí Teaghlaigh Éireann

8th May 2012

Prof Richard Costello
Dept of Medicine
RCSI Smurfit building
Beaumont Hospital
Dublin 9

Inhaler Adherence in real life General Practice

Dear Prof Costello,

I wish to confirm that on review of your amendments I am now happy to approve your study.

Yours sincerely,

Prof Colin Bradley
Chair Research Ethics Committee

5 Lincoln Place, Dublin 2
Tel: (01) 676 3705/06 Fax: (01) 676 5850
e-mail: info@icgp.ie www.icgp.ie

Regd. in Ireland at the Irish College of General Practitioners Limited
Registration Number 100456

Kieran Ryan
Chief Executive Officer

APPENDIX 2: Journal publications.

See RCSI Institutional Repository