PERFORMANCE OF TWO DIFFERENT TYPES OF INHALERS: INFLUENCE OF FLOW AND SPACER ON EMITTED DOSE AND AERODYNAMIC CHARACTERISATION

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LIST OF ABBREVIATIONS

ACI	Andersen cascade impactor
API	active pharmaceutical ingredient
AUC	area under the curve
AVG	average
BP	British Pharmacopoeia
CFC	chlorofluorocarbons
CFC-BDP	chlorofluorocarbon beclomethasone
CI	confidence interval
C_{max}	maximum plasma concentration
COPD	chronic obstructive pulmonary disease
DPIs	dry powder inhalers
CPMP	Committee for Proprietary Medicinal Products
EMEA	European Medicines Evaluation Agency
FDA	Food and Drug Administration
FEV_1	forced expiratory volume in one second
FPD	fine particle dose
FPF	fine particle fraction
FPFN	fine particle fraction nominal dose [%]
FVC	forced vital capacity
g	gram
GM-CSF	granulocyte-macrophage colonystimulating factor
GSD	geometric standard deviation
HFA	hydrofluorocarbon
HFA-BDP	hydrofluoroalkane-134a beclomethasone
HPLC	high performance liquid chromatography

ICH	International Committee on Harmonisation
ICRP	International Commission on Radiological Protection
ICS	inhaled corticosteroids
IV	intravenous
JVP	jugular venous pressure
kPa	kilopascal
L	Litre
LD	laser diffractometry
L/min	litre per minute
LOD	limit of detection
LOQ	limit of quantification
MDI	metered dose inhaler
mg	milligram
mg/L	milligram per litre
mg/ml	microgram per millilitre
min	minute
ml	millilitre
ml/min	millilitre per minute
mM	millimolar
MMI	Marple Miller cascade impactor
MMAD	Mass median aerodynamic diameter
MOC	Micro orifice collector
MSLI	multi-stage liquid impinger
ng	nanogram
ng/ml	nanogram per millilitre
nm	nanometer
NGI	next generation impactor

OPCS	optical particle counters
PDA	phase Doppler particle size analysis
PEF	peak expiratory flow
PhEuro	European Pharmacopoeia
PIF	peak inspiratory flow
PK	pharmacokinetic
pKa	dissociation constant
pMDIs	pressurised metered dose inhalers
R^2	correlation coefficient
RR	respiratory rate
RSD	relative standard deviation
SSGI	single-stage glass impinger
STDEV	standard deviation
sec	second
TOF	time-of-flight aerodynamic
SPECT	single photon emission computed tomography
TDPS	total dose per shot
USP	United States Pharmacopoeia
UV	ultraviolet
v/v	volume per volume
v/w	volume per weight
w/w	weight per weight
λ	wavelength
°C	degree centigrade celsius
µg	microgram
µl	microlitre
µm	micrometre

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DEDICATION

This thesis is dedicated to my parents, who have been a constant source of encouragement and inspiration to me throughout my life. Thank you for all the unconditional love, guidance and support that you have always given me. Although my father is not here to give me strength and support I always feel his presence which used to urge me to strive to achieve my goals.

I wish to extend this dedication to my wife, Fouziyah Alsrhed, who has supported my desire to further my education and remains willing to engage with the struggle and ensuing discomfort associated with being a postgraduate student's partner. Only with her unflinching love and support has this work been accomplished. I also dedicate this to my delightful children, Abdullah, Salem and Haya, who have tolerated my absences and been so supportive even when being 'without Dad' was hard.

This thesis is also dedicated to my brothers and sisters who have given me unlimited support. A very special thanks to my oldest brother, Saad, who has supported me all the way since the beginning of my studies and without whose guidance and gentle prodding, I would still be trying to decide on a major for my undergraduate course.

ABSTRACT

This thesis is based around examination of three mainstream inhaled drugs Formoterol, Budesonide and Beclomethasone for treatment of asthma and COPD.
The areas investigated are these which have been raised in reports and studies, where there are concern, for drug use and assessment of their use.
In reporting this work the literature study sets out a brief summary of the background and anatomy and physiology of the respiratory system and then discuses the mechanism of drug deposition in the lung, as well as the methods of studying deposition and pulmonary delivery devices. This section includes the basis of asthma and COPD and its treatment. In addition, a short section is presented on the role of the pharmacist in improving asthma and COPD patient's care.
Therefore the thesis is divided into 3 parts based around formoterol, budesonide and beclomethasone.
In the first case the research determines the *in-vitro* performance of formoterol and budesonide in combination therapy. In the initial stage a new rapid, robust and sensitive HPLC method was developed and validated for the simultaneous assay of formoterol and the two epimers of budesonide which are pharmacologically active.
In the second section, the purpose was to evaluate the aerodynamic characteristics for a combination of formoterol and the two epimers of budesonide at inhalation flow rates of 28.3 and 60 L/min. The aerodynamic characteristics of the emitted dose were measured by an Anderson cascade impactor (ACI) and the next generation cascade impactor (NGI). In all aerodynamic characterisations, the differences between flow rates 28.3 and 60 were statistically significant in formoterol, budesonide R and budesonide S, while the differences between ACI and NGI at 60 were not statistically significant.
Spacers are commonly used especially for paediatric and elderly patients. However, there is considerable discussion about their use and operation. In addition, the introduction of the HFAs propellants has led to many changes in the drug formulation characteristics. The purpose of the last section is to examine the performance of different types of spacers with different beclomethasone pMDIs. Also, it was to examine the hypothesis of whether the result of a specific spacer with a given drug/ brand name can be extrapolated to other pMDIs or brand names for the same drug.
The results show that there are different effects on aerodynamic characterisation and there are significant differences in the amount of drug available for inhalation when different spacers are used as inhalation aids. Thus, the study shows that the result from experiments with a combination of a spacer and a device cannot be extrapolated to other combination.

KEY WORDS

Dry powder inhalers (DPIs) turbuhaler®, pressurised-metered-dose-inhalers (pMDIs), breath-operated devices, hydrofluoroalkane (HFA), chlorofluorocarbon (CFC), formoterol, budesonide R, budesonide S,

beclomethasone, dose emission, aerodynamic analysis, valved holding chambers, metering performance, spacers,

LIST OF PUBLICATIONS

Sections of this thesis have already been published in the following form:

ALMEZINY, M. and CLARK, B. (2007) High performance liquid chromatography assay method for simultaneous quantitation of formoterol and the two epimers of budesonide. JOURNAL OF PHARMACY AND PHARMACOLOGY, 59, 74.

CHAPTER 1

LITERATURE REVIEW

1 LITERATURE REVIEW

ANATOMY AND PHYSIOLOGY OF THE RESPIRATORY SYSTEM

1.1.1 Introduction

In order to understand the therapeutic application of drugs in this area, it is firstly important to discuss the respiratory system. The main function of a respiratory system is gas exchange and, at the same time, it plays a role in maintaining the acid-base balance by removing CO_2. Also, the respiratory system participates in phonation by moving the air through the vocal cords (2007). The respiratory tract can be classified into two main divisions, the upper and lower airways, as shown in Figure 1.1 (Clarke et al., 1984). The upper airway consists of the nasal cavity, oral cavity and pharynx. The pharynx includes three parts: nasopharynx, oropharynx and laryngopharynx. The upper respiratory tract conducts, humidifies and warms air. Also, it takes part in swallowing, smelling and speaking. Furthermore, it helps to filter the air from large particles by impaction in the nose and oropharynx (Packet, 2005).

The lower airway includes the larynx, the trachea, the bronchi and the lungs. The main function of the lower airway is conduction of air. The majority of the respiratory tract is lined with ciliated cells which brush mucus and debris up and out of the tracts.

1.1.2 Lung

The lungs are paired, cone-shaped organs which take up most of the space of the thorax. The right lung is larger than the left lung. The two lungs are each covered within a double membrane known as the pleura. The lungs are

divided into lobes by invaginations of the pleura, which for most of the time are incomplete. The right lung consists of three lobes, while the left lung

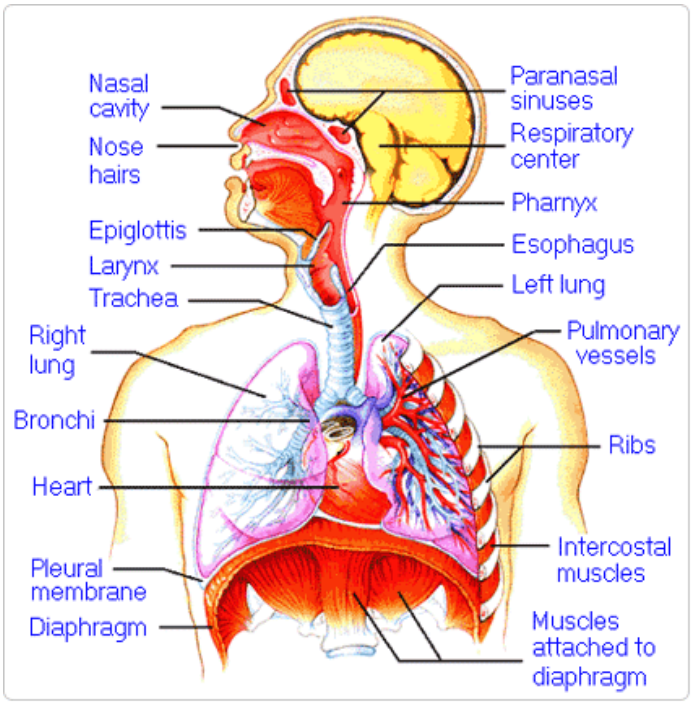

Figure 1.1 Respiratory System: structure detail Source: (AHA, 2006)

consists of two lobes, (see Figure 1.2) (Clark et al., 2005). The upper lobe lies in front of the lower lobe. The lobes further separate into the bronchopulmonary segments by fibrous septa that expand inwards from the pleural surface, each of which has a segmental bronchus. The bronchopulmonary segment is subdivided into lobules about 1 cm in diameter and has a pyramid shape, with the apex facing towards the bronchioles that, supply them. Within each lobule a terminal bronchus supplies an acinus, and within this structure further separation of the bronchioles finally gives rise to the alveoli (Clark et al., 2005).

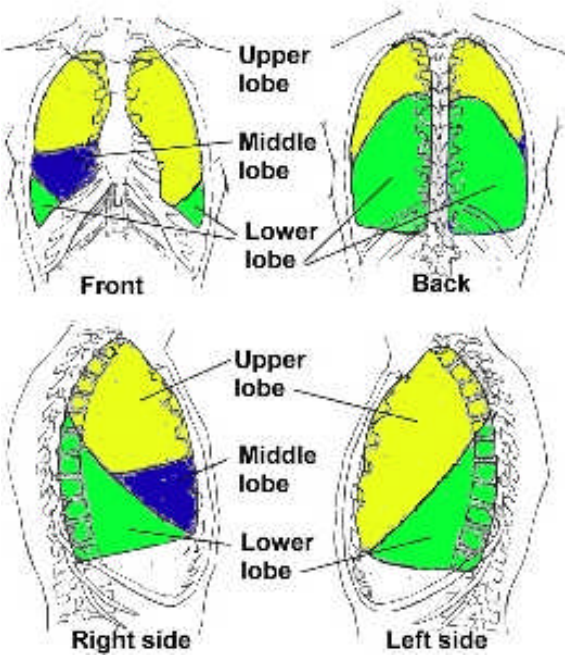

Figure 1.2 Anatomy of the lungs showing the different lobes on the right and left side Source: (YourSurgery.Com, 2009)

1.1.3 Trachea, bronchi and bronchioles

The trachea is a tubular structure located close to the sixth cervical vertebra. Its length is 10 to 15 cm by 16 to 20 cm width, in a horseshoe shape. The trachea, under the junction of the manubrium sternum and the second right costal cartilage, branches at the carina into the right and left main bronchi (Figure 1.3). In the same way, the right main bronchus branches again into the upper lobe and the intermediate bronchus, then each lobe is separated into the middle and lower lobe bronchi. On the other hand, the left main bronchus is separated into upper and lower lobe bronchi only. Further additional divisions occur as each lobar bronchus divides into segmental and subsegmental bronchi. At the end of the terminal bronchioles are the alveoli (Clark et al., 2005).

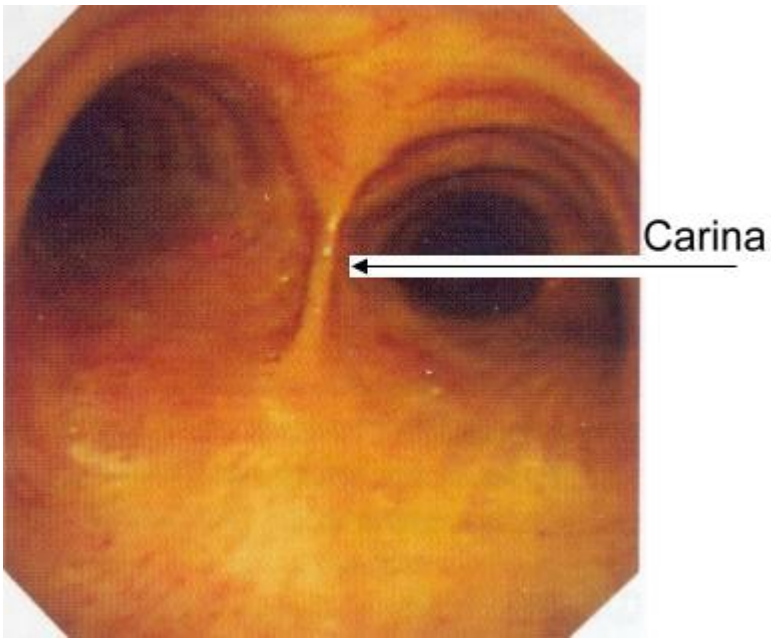

Figure 1.3 The carina where the trachea branch to the left main and right main bronchi Source: Adapted from (Selby, 2002).

In total, there are about 23 divisions between the trachea and the alveoli, with the structure of the tubes changing progressively from the trachea to the terminal bronchioles, as shown in Figure 1.4 (Levitzky, 2007). The structures of the first seven divisions comprise walls of cartilage and smooth muscle. Also, the structure has an epithelial lining with cilia and goblet cells. In addition, it has mucus-secreting endocrine cells as either Kulchitsky or amine precursor and uptake decarboxylation (APUD) containing 5-hydroxytryptamine (Levitzky, 2007).

The divisions from 16 to 18 have no cartilage. Compared to the first seven divisions, there is a progressively thinner muscular layer, a single layer of ciliated cells, and fewer goblet cells, as well as granulated Clara cells that produce a surfactant-like substance.

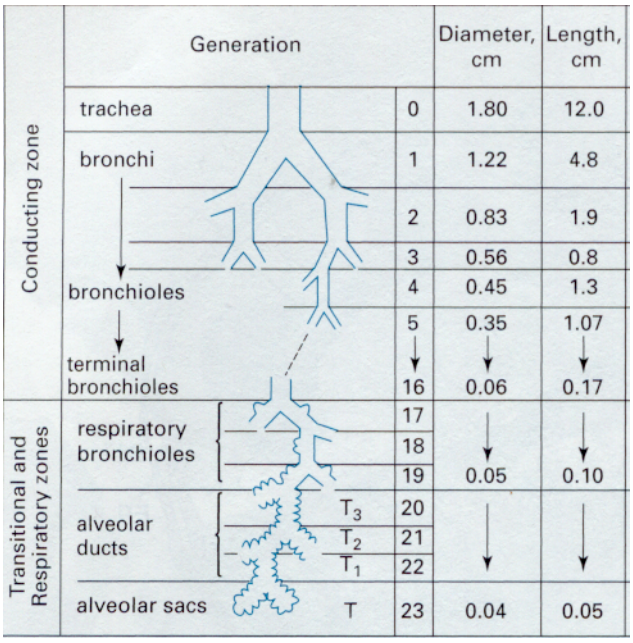

Figure 1.4 Schematic representation of airway branching in human lung with approximate dimensions Source: (Wiebel, 1984)

The ciliated epithelium is an important defence mechanism. Every cell contains around 200 cilia beating at 1000 beats per minute in well controlled waves of contraction (Clark et al., 2005). Furthermore the trachea has many mechanical and chemical receptors. The muscles of the posterior tracheal wall contain slowly adapting pulmonary stretch receptors (SARs) which are lung vagal afferents which play a role in controlling breathing pattern and airway smooth muscle tone. They also create dilation of the upper airway through reducing vagal efferent action. In addition, the rapidly adapting irritant receptors are around the tracheal circumference. These can be considered as cough receptors. Also, the tracheal circumference contains other reflex action such as bronchoconstriction (Rajagopal et al., 2005).

1.1.4 Pleura

The intact visceral pleura is a thin translucent sheet of mesothelial tissue. It is the surface covering of the lung and lines the interlobar fissures. It is contiguous at the hilum with the parietal pleura. The parietal pleura is the surface which covers the chest wall. A small volume of fluid fills the pleural space, ranging from 1 to 20 mL. The factors controling the movement of fluid into and out of the pleural space are hydrostatic, colloid osmotic and tissue pressure in the parietal and visceral pleura. The parietal pleura includes lymphatics that drain into the internal mammary artery, periaortic arteries, and diaphragmatic lymph nodes (Clark et al., 2005, Crapo, 2004). In order to prevent lung collapse, the pressure within the pleural space i.e. (between visceral and parietal pleural surfaces) is usually maintained at sub-atmospheric pressure (Warrell, 2003)

1.1.5 Alveoli

The alveoli are the final branching of the respiratory tree and perform gas exchange for the lung. Each lung contains about 300 million alveoli, representing a total surface area of approx. 40-80 m^2. There are two types of alveolar epithelial cells. The predominant type I cells pneumocytes which have an extremely attenuated cytoplasm and provide a thin barrier to allow a rapid gas exchange. Furthermore, Type I cells are linked together by tight junctions that reduce the fluid movements in and out of the alveoli (Bourke, 2003, Clark et al., 2005). The other is Type II pneumocytes which cover less of the epithelial lining and are at the borders of the alveolus and contain distinctive lamellar vacuoles, which produce the surfactant. The pores of

Kohn are apertures in the alveolar septum, which allow the communication of two adjacent alveoli (Clark et al., 2005).

1.1.6 Muscles of respiration

The muscles of respiration can be classified into two categories. The first is the inspiratory muscles which include the diaphragm, the external intercostals and the accessory muscles of inspiration. The second category is the expiratory muscles which include the abdominal muscles and internal intercostal muscles (Levitzky, 2007).

1.1.6.1 Diaphragm

This is considered as the primary inspiration muscles, which separate the abdominal and the thorax cavities. Nevertheless, its involvement in the expiratory is limited. It has a large surface area about 250 cm^2 and is dome-shaped (Levitzky, 2007). The parietal pleura covers the upper part while the peritoneum covers the lower part. The muscle fibres begin from the lower ribs and insert into the central tendon (Clark et al., 2005).

1.1.6.2 External intercostals.

Their contraction leads to rib cage enlargement.

1.1.6.3 Accessory muscles of inspiration

Usually, they do not participate in normal quiet breathing. However, they can be involved in high demand situations such as in exercise, in coughing or sneezing, or in asthma (Levitzky, 2007).

1.1.7 Pulmonary vasculature and lymphatics

The right ventricle pumps deoxygenated blood to the pulmonary arteries, which branch into the pulmonary capillaries, which surround the alveoli, for gas and fluid exchange. The pulmonary venules drain into four main pulmonary veins carrying the blood back to the left side of the heart.

Lymphatic channels are located in the interstitial space between the alveolar cells and the capillary endothelium of the pulmonary arterioles (Clark et al., 2005).

1.1.8 Physiology of areathing, coughing and sneezing

During inspiration, the size of the thorax is enlarged by the movement of the diaphragm downward and the lower ribs upward and outward. As a consequence, the thoracic size will be increased and the pressure within the chest decreases. As a result, air moves into the lungs from the atmosphere. In contrast, the expiration is a passive process but becomes active during exercise, speech, sneezing or coughing and during bronchitis. During expiration, the diaphragm and other respiratory muscles relax, thus decreasing the size of the thorax, pressure then increases and air moves out of the chest into the atmosphere, as shown in Figure 1.5 (Selby, 2002)

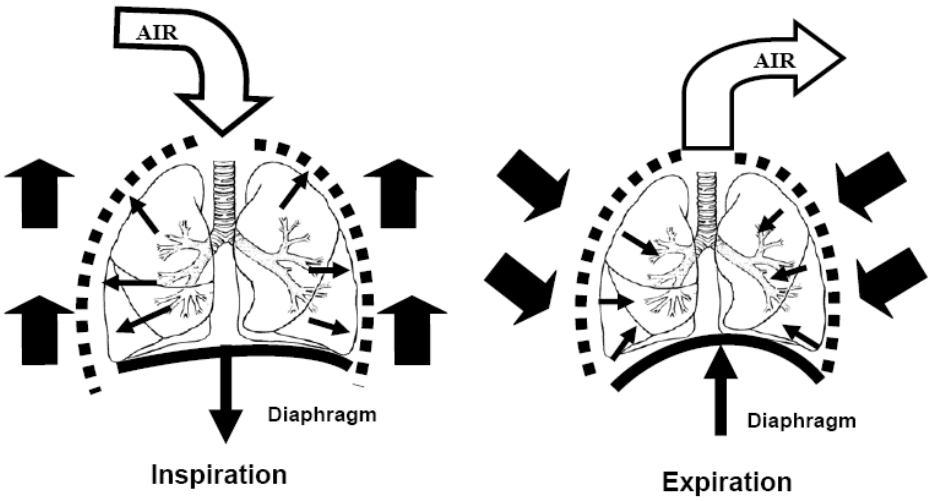

Figure 1.5 An illustration for inspiration and expiration Source: (Packet, 2005).

1.1.9 Regulation of ventilation

Breathing is spontaneously initiated in the brainstem in the central nervous system. It receives its information from mechanoreceptors and chemoreceptors. Mechanoreceptors include stretch, irritant and juxtacapillary (J) receptors. Stretch receptors are sensitive to stretch and movement in the respiratory system. Irritant receptors react to stimulation of inhaled irritants by generating bronchoconstriction and hyperpnoea. These receptors are triggered by congestion of pulmonary capillaries and increases in interstitial fluid volume, which stimulate rapid, shallow breathing. Chemoreceptors are located centrally, peripherally and within the lung tissue. They are also sensitive to pH which decreases due to CO_2 increase. While peripheral chemoreceptors are located in the aortic arch and carotid body, they respond to hypoxemia (Selby, 2002, Levitzky, 2007). Breathing generally is involuntary, however, there is ability for voluntary control such as speech and exercise. There are many factors which can depress the respiratory muscle

function, including drugs such as benzodiazepines and opioids. Depression is also caused through ingestion of ethanol and electrolyte disturbance, particularly potassium, magnesium and phosphate, systemic acidosis and neuromuscular disease (Figure 1.6) (Selby, 2002).

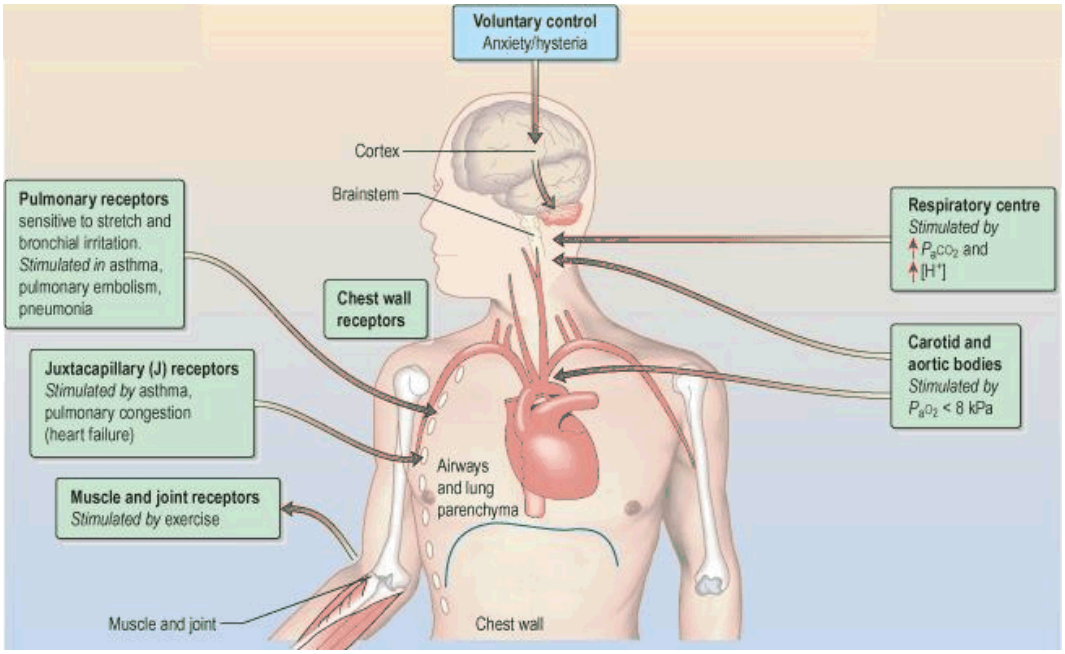

Figure 1.6 Chemical and neurogenic factors in control of ventilation Source: (Clark et al., 2005).

Coughing is a defence mechanism to protect the respiratory system by expelling secretions and foreign bodies from the lower respiratory tract. It is activated by stimulating the rapidly adapting irritant receptors. (Rajagopal et al., 2005, Selby, 2002). Cough can be triggered by many causes including asthma, postnasal drip, gastroesophageal reflux (GERD), an over-production of mucus due to many diseases or an adverse effect of drugs. A typical example is the pharmacological group of angiotensin II converting enzyme inhibitors (ACE-I) (Levitzky, 2007). Another defence mechanism is sneezing, activated by stimulating receptors located in the nose and nasopharynx (Levitzky, 2007).

LUNG DEPOSITION

1.1.10 Introduction

For the treatment of respiratory disorder, different types of inhaler are used. Drug particles are deposited in the respiratory system depending on the drug physical and chemical properties and the host's physiology.

1.1.11 Aerosol

Stuart, (1973) defined an aerosol as "any system of solid particles or liquid droplets of sufficiently small diameter to maintain some stability as suspension in air". It can be classified into monodisperse, where the particles have approximately the same size and heterodisperse or polydisperse when different sizes are involved. But the perfect monodisperse system does not exist and it is widely accepted that if an RSD < 20% p/p, an aerosol can be called monodisperse (Newman et al., 1982).

1.1.12 Mechanism of deposition

Although there are many mechanisms for lung deposition only three mechanisms are important: inertial impaction, gravitational sedimentation, and Brownian diffusion (Figure 1.7) (Newman et al., 1982, Bisgaard et al., 2002).

1.1.12.1 Inertial Impaction.

The depositions of the majority of drug particles larger than a few μm occur by inertial impaction. When the particles are heavy or travelling at high speed, this may lead to the particles being unable to follow a change in

direction and as a result they will impact on the airway wall (Bisgaard et al., 2002, Newman et al., 1982).

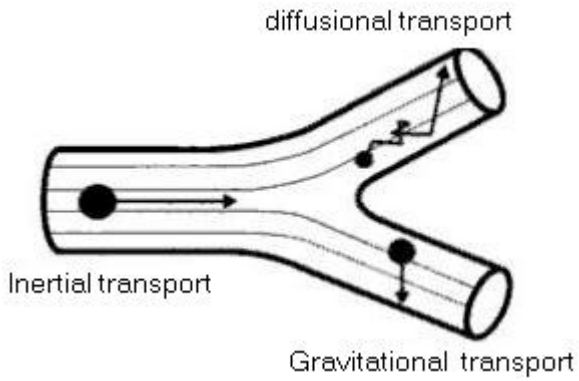

Figure 1.7 Illustration of particle transport onto airway surfaces Source: (Bisgaard et al., 2002).

1.1.12.2 Gravitational sedimentation

Particle sedimentation is driven by the gravitational force which is balanced by air resistance. Particle sizes range from 0.5 μm to 5 μm and may travel to peripheral parts of the lung where they can settle onto smaller airways. This can occur during quiet breathing or breath holding (Newman et al., 1982, Bisgaard et al., 2002).

1.1.12.3 Brownian diffusion

For particles smaller than 0.5 μm, Brownian diffusion is the most important mechanism of deposition. Here, particles inside the airways may be displaced by the random bombardment of gas molecules which impact on the airway walls (Bisgaard et al., 2002, Newman et al., 1982).

1.1.12.4 Insignificant mechanisms

These include electrical charge force, diffusophoresis, thermophoresis and simple contact. The particles are deposited by electrical force between the particles and the airway walls since pharmaceutical formulations are usually not strongly charged. As a result, this mechanism can play an insignificant role (Bisgaard et al., 2002). Diffusophoresis is the diffusion of gas from the area of high to low concentration. In the same way, thermophoresis is the movement of particles from regions of high to low temperature. When the particles are larger compared with the airway diameter, simple contact may result in deposition of the particle (Newman et al., 1982, Bisgaard et al., 2002).

1.1.13 Summary of mechanism of deposition

In summary, the parameters which most affect particle transport into the respiratory tract are the particle size, density, velocity and time. It is a common practice to substitute the breathing cycle period for time and flow rate for velocity as shown in Table 1.1.

Table 1.1 Summary of effects of four parameters on deposition mechanisms

Mechanism of deposition	Particle size	Particle density	Breathing cycle period	Flow rate
Inertial	Increase	Increase	Independent	Increase
Gravitational	Increase	Increase	Increase	Independent
Brownian diffusion	Decrease	Independent	Increase	Independent

1.1.14 Concept of aerodynamic particle diameter

The particle size of an aerosol is a crucial physical property affecting the lung drug deposition. In addition, aerodynamic diameter rather than geometric diameter controls particle deposition in the lungs. The aerodynamic diameter is a product of geometric diameter and the square root of density as shown in this equation ($d\sqrt{p}$), where *p* is the particle density and d the geometric diameter. Furthermore, particles with the same product of ($d\sqrt{p}$)will exhibit identical deposition. On the other hand as a particle becomes more porous, it becomes less dense and as result the aerodynamic diameter decreases. Accordingly, as density decreases, particles that are larger in geometric diameter can deposit deeper into the lung region, because they are smaller in aerodynamic diameter (Mandal, 2005)

Bisgaard and co-workers (2002) defined the aerodynamic diameter of particles as the diameter of a fictitious sphere of unit density which, under the action of gravity, settles with the same velocity as the particles in the equation.

For spheres at the same velocity, they behave with aerodynamically the same deposition, although it should be noted that this concept is limited to particles transported by gravitational and inertial transport (Bisgaard et al., 2002).

METHODS OF STUDYING DEPOSITION

There are many methods to identify drug deposition in the lung two main types: *in-vivo,* such as the pharmacokinetic and scintigraphic methods and *in-vitro,* methods which have potential value in predicting lung deposition. In

addition, they have a major role in the quality control for inhaled formulations, examples are dose emission and particle size distribution

The information that is available from these *in-vivo* and *in-vitro* studies includes total lung dose, extrapulmonary delivery, drug distribution within the respiratory system, the relationship between lung dose and therapeutic effect and the influence of factors such as disease, inhalation technique and intra- and inter-patient variability (Bisgaard et al., 2002).

1.1.15 *In-vivo* methods

1.1.15.1 Introduction

In many devices, after inhalation, up to 20% of the inhaled dose is delivered to the lung and 80% is deposited in the oropharyngeal region and therefore is swallowed. The fraction delivered to the lung is cleared either by the mucociliary ladder and swallowed within 24 hours or by absorption through the very large surface area of the respiratory system into systemic circulation, as shown in Figure 1.8 (Chrystyn, 2001).

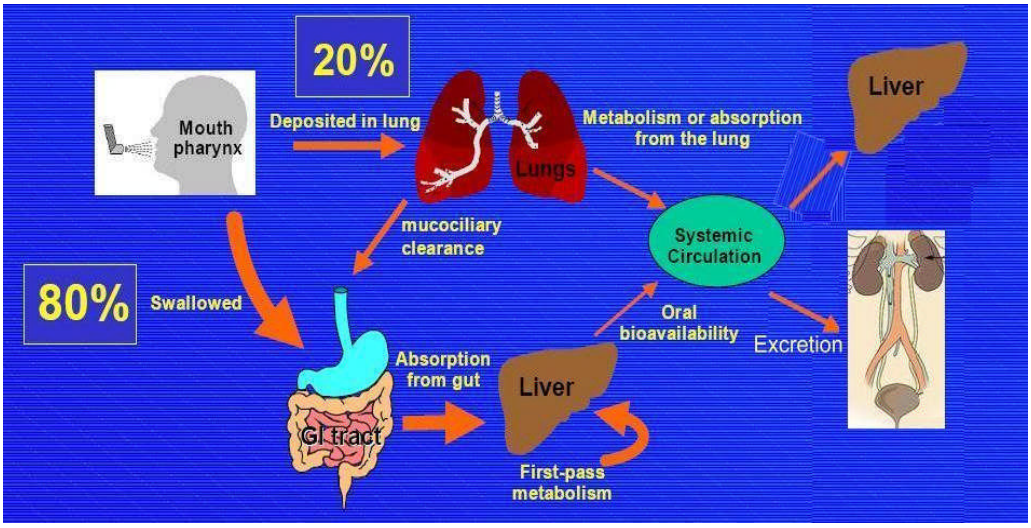

Figure 1.8 Fate of inhaled drug

The drug proportion which reaches the systemic circulation has the potential to cause extra-pulmonary adverse effects such as in the case of corticosteroid osteoporosis and Cushing's syndrome. However, when the corticosteroid is absorbed from the gut it undergoes first-pass metabolism and these adverse effects are minimised. Corticosteroids are generally highly affected by first-pass metabolism. As an example, budesonide is metabolised up to 89%, fluticasone > 99% and mometasone >99% (O'Connell, 2003).

In recent years, as the pharmaceutical development of new drugs through the inhalation route has evolved, interest in the *in-vivo* assessment of drug delivery to the lung has increased. But the results from early studies have been variable. The selection of subjects has had a major influence on the validity of the study results. Also, in the early studies, the number of subjects was often relatively low. Added to this, the studies were often conducted under well-controlled conditions and these generally do not fully reflect normal patient conditions. Therefore, it is possible to suggest that the results obtained using well trained, supervised healthy subjects and under ideal conditions, do not always reflect the real situation. With actual patients, many factors often have a greater impact on the results; these include airflow obstruction and inhalation technique (Bisgaard et al., 2002). As a result, it is suggested that considerable caution should be taken when extrapolating data from healthy well trained subjects to real patients (Derendorf et al., 2001).

1.1.15.2 Imaging

There are three main methods for imaging, planar gamma scintigraphy, single photon emission computed tomography (SPECT) and positron emission tomography (PET). The main advantage of these imaging

techniques over others is the ability to localise deposition within the body, including extrapulmonary, and the distribution throughout the airways. However, there are safety issues all imaging methods which use radionucleotides expose the subjects to health risks, more pronounced in children than in adults (Bisgaard et al., 2002).

A typical example is where the drug is labelled with a gamma ray-emitting isotope and a gamma camera is used to image radiation emitting from the radioisotopes. An illustration of the general process used in aerosol imaging is summarised in Figure 1.9.

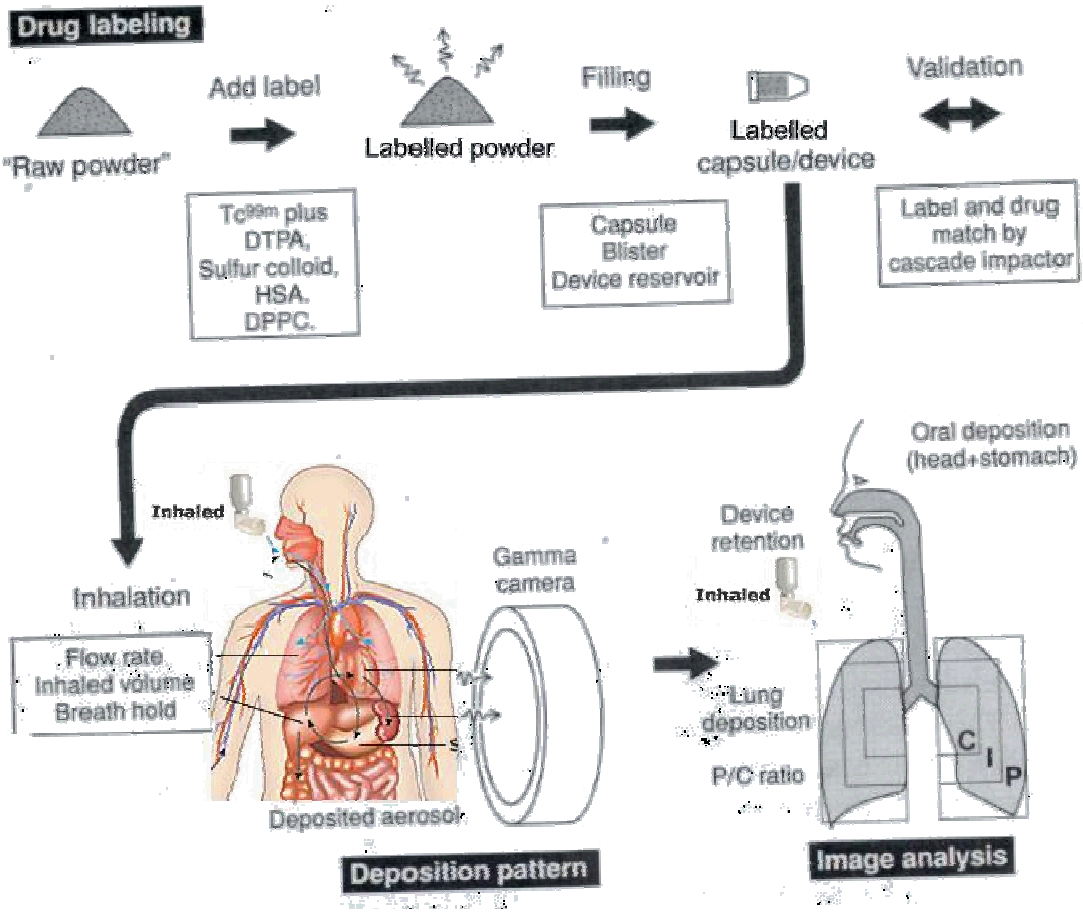

Figure 1.9 Schematic illustration of application of gamma scintigraphy to estimate lung deposition Source: Adapted from (Bisgaard et al., 2002).

In practice, imaging techniques are subject to a number of operational challenges, including labelling of the drug formulation and interpretation of the images produced.

1.1.15.3 Relationship between drug delivery and effect (pharmaco-dynamics).

It has been shown that a good relationship exists between lung deposition and the effects for both bronchodilators and steroids (Derendorf et al., 2001). In addition, some research has shown a relationship between the pattern of deposition and the pharmacodynamic effect, especially for inhaled steroids. There has, however, been limited work in this area because the therapeutic effect of inhaled steroids needs weeks to be seen. For β_2 agonists, the reason is that doses are usually administered at close to/or supermaximal level and for that reason the doses are close to the plateau of the dose response curve (Bisgaard et al., 2002).

1.1.15.4 Pharmacokinetic methods in use

Pharmacokinetic methods are used to evaluate the lung deposition, although they do not generally provide information on the distribution of drug into different regions of the lungs (Derendorf et al., 2001). These methods estimate total systemic delivery via oral and inhaled routes by means of area under the curve (AUC) data or urinary excretion of the drug (Chrystyn, 2001). Historically, the pharmacokinetic methods have faced difficulties because of small amounts of drug present in the systemic circulation. Today, this problem has been overcome by using more sensitive analytical techniques. The pharmacokinetic techniques provide the advantage over imaging

techniques of avoiding the use of radiation and the drug formulation can be used without any modifications.

As shown in Figure 1.8, in some inhaled drugs, a considerable amount may be absorbed from the gastrointestinal route. Therefore, to distinguish between the inhaled and oral absorption routes, many methods have been suggested. One example is to block gastrointestinal absorption by activated charcoal. The second is to collect the serum or urine sample within a specific time, using the time lag between the pulmonary and gastrointestinal absorptions. Thirdly, the amount of drug in serum can be measured directly when the gastrointestinal absorption is negligible, e.g. sodium cromoglycate, first-pass metabolism is very high, e.g. fluticasone, or by correction for drugs of known bioavailability (Derendorf et al., 2001, Bisgaard et al., 2002).

1.1.15.4.1 Identification of total systemic delivery

In the case where the systemic availability through gastrointestinal absorption is negligible, e.g. fluticasone and sodium cromoglycate, all systemic drug concentrations represent the absorption from pulmonary routes (Derendorf et al., 2001).

1.1.15.4.2 Assessment of pulmonary deposition using activated charcoal.

For drugs where gastrointestinal absorption involvement is considerable, block of the absorption is necessary and a solution is to use activated charcoal administered together with the drug, as shown in Figure 1.10.

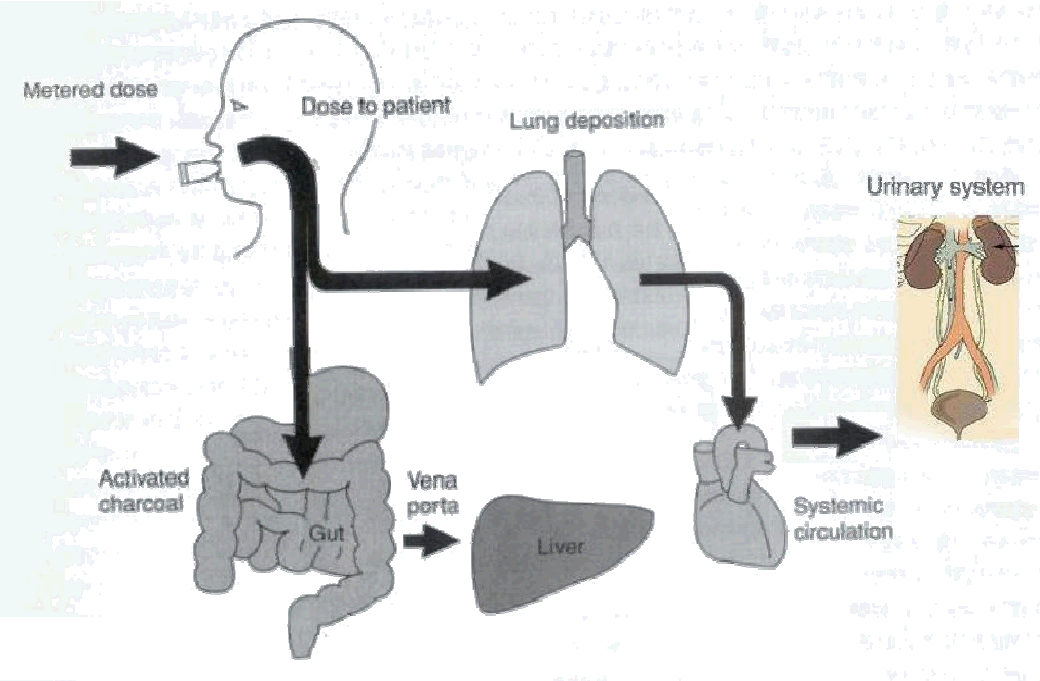

Figure 1.10 Schematic illustration of the application of pharmacokinetic and the charcoal block methods to estimate the lung deposition Source: Adapted from (Bisgaard et al., 2002)

1.1.15.4.3 Assessment of pulmonary deposition utilizing lag time.

The absorption of most drugs occurs in the small intestine which takes time. On the other hand, the absorption from the pulmonary system is rapid and this leads to a time lag between the absorptions from the two systems. As a result samples for pulmonary deposition are collected in this lag time (Chrystyn, 2001).

1.1.15.4.4 Correction for drugs of known bioavailability

However many methods have also used activated charcoal to measure pulmonary deposition. But as an alternative, some clinical studies have used oral bioavailability data. In practice this technique is limited to drugs whose oral bioavailability has been well established.

1.1.16 *In-vitro* methods

1.1.16.1 Introduction

In-vitro methods contribute in a major way to drug development in the pharmaceutical industry. Alongside this, researchers use *in-vitro* methods to predict the drug deposition in lung. The reason is that pharmacological effects of an inhaled drug have a good relation with the amount of drug depositing in the lower airways and the deposition pattern, as shown in Figure 1.11. In addition, *in-vitro* methods are used to determine the difference between different inhaled formulations (Van Oort, 1995).

A number of methods have been reported to characterise the particle size of a drug. In practice, it is possible to broadly categorise these into two areas: optical and inertial methods.

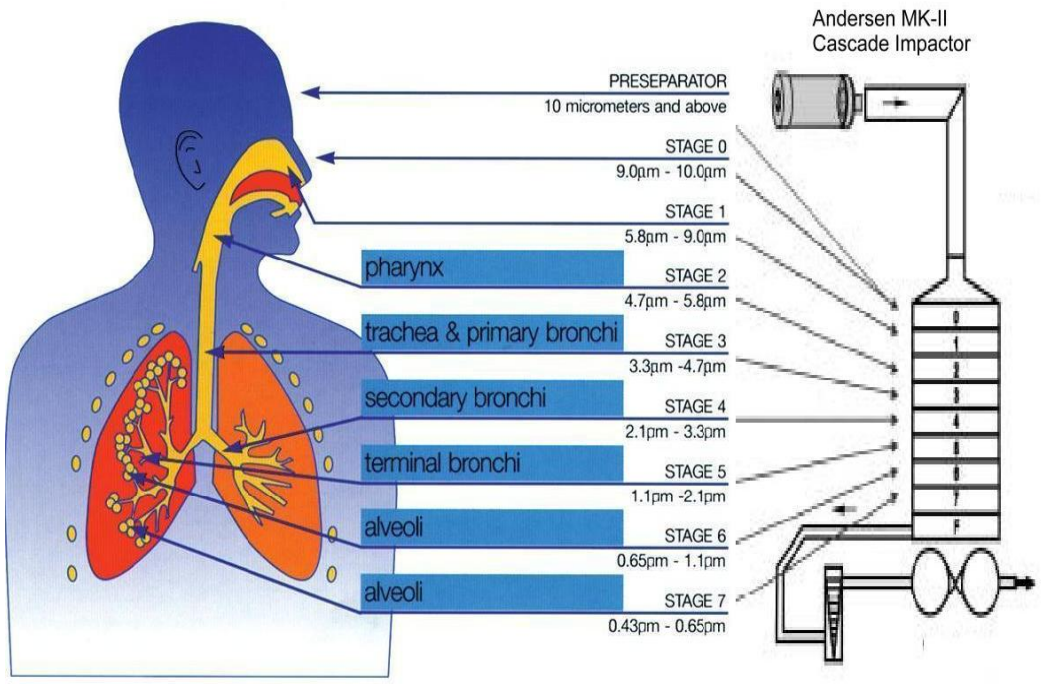

Figure 1.11 Relation between Andersen 8-stage CI cut size at 28.3L/min and likely deposition in lung. Source: Adapted from (Mitchell et al., 2000)

1.1.16.2 Optical methods

Optical methods include microscopy, time-of-flight aerodynamic particle size analyser (TOF), light interaction methods optical particle counters (OPCS), laser diffractometry (LD) and phase Doppler particle size analysis (PDA). These instruments provide rapid techniques and, in addition, the TOF instrument measures the aerodynamic particle size. But they do have the weakness that they are not drug-specific and lack the capability of a direct assay for the active pharmaceutical ingredient (API), because they are unable to differentiate between drug particles and carrier particles.

1.1.16.3 Inertial impaction method

The inertial cascade impaction is the gold standard to determine the aerodynamic characteristic of emitted dose. Generally, it is the method most acceptable to the regulatory agencies as it is based on the inertial impaction concept. Since the inhaled formulations comprise a combination of API and other excipients, it is important to measure the API. The method also uses the entire dose as sample and is able to measure the aerodynamic size. The disadvantage is that it is calibrated only at fixed flow rates.

The principle of cascade impactor operation is based on inertial impaction. Each stage of the impactor contains one or more nozzles or jets through which the sample loaded air stream is drawn, directing any particles towards the surface of the collection plate for that specific stage, as shown in Figure 1.12. The determining factor of whether a particular particle impacts on that stage is its aerodynamic size. Particles with sufficient inertia will impact on that particular stage collection plate, while smaller particles with insufficient inertia will remain in the air stream and pass to the next stage. As the jets get

smaller, the air velocity increases and smaller particles are collected. Finally, the smallest particles are collected on an after-filter. However, the influence of gravity becomes more observable at low flow rates with impactors (Mitchell et al., 2000).

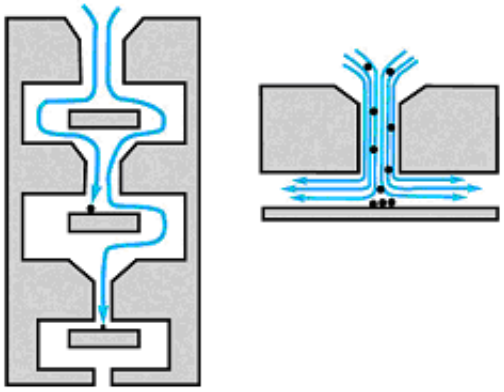

Figure 1.12 Principle of operation of cascade impactors Source: (Copley, 2007).

The collection efficiency curve is the most important characteristic of each stage of a cascade impactor which is a measure of the percentage of particles of a specific size collected at the impaction plate of that stage. In an ideal situation, this curve should have a sharp straight line between the size of particles collected and those which are not. In the real world, however, this curve has an `S' shape as shown in Figure 1.13 (Copley, 2008a).

Inertial impaction methods are divided into cascade impactors and impingers. The difference between the two methods is that the collection substrate is a solid surface, whereas impingers use a liquid to collect the particles. The impingers are calibrated at 60L/min and are easy to use (Van Oort, 1995).

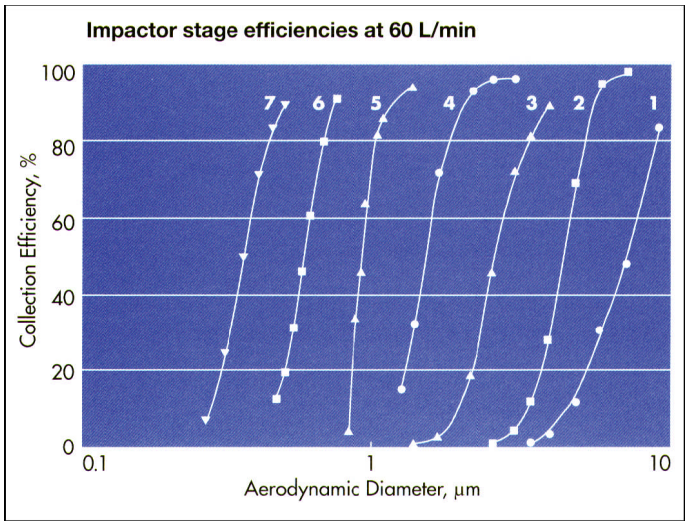

Figure 1.13 Impactor stage efficiencies at 60 L/Min. Source: (Copley, 2007).

One of the main problems with cascade impactors is carry-over of particles to smaller collection stages. This can take place due to re-entrainment into the airstream or due to particle bounce at the collection stages. In order to minimise these effects, suitable coating of the collection stages may be necessary, especially, in the case of DPIs and certain MDIs (Mitchell et al., 2000).

It is also important to note that particle size analysis by cascade impactor can be interrupted by many factors which can affect the accuracy of the results as summarised in Table 1.2.

The European Pharmacopoeia (PhEuro) lists the following apparatus for aerodynamic assessment of fine particles in both MDIs and DPIs:

- Apparatus A: Single-Stage Glass Impinger (SSGI)
- Apparatus C: Multi-Stage Liquid Impinger (MSLI)
- Apparatus D: Andersen Cascade Impactor (ACI)
- Apparatus E: Next Generation Impactor (NGI)

Methods for Apparatus A are also specified for nebulisers (PhEuro, 2007). On the other hand, the United States Pharmacopeia lists six impactors which can be used for aerodynamic size distribution (USP, 2005) :

- Apparatus 1 for MDIs: ACI.
- Apparatus 2 for DPIs: MMI.
- Apparatus 3 for DPIs: ACI + Preseparator.
- Apparatus 4 for DPIs: MSLI.
- Apparatus 5 for DPIs: NGI + Preseparator.
- Apparatus 6 for MDIs: NGI.

Table 1.2 Potential causes of error in Impactor-based particle size measurements Impactor-related issues Source: (Mitchell et al., 2000)

Factor	Potential Influence on Particle Size Distribution Accuracy
Correct location of collection surfaces	High
Proper accounting for collection surfaces and back-up filter	High
Assertion of stage order	High
Air leakage into impactor	Low, unless leak is massive
Poor seal and orientation between induction port/impactor or between induction port/pre-separator/impactor	Low, unless leak is massive or components grossly out of alignment
Inadequate liquid volume or liquid missing from collection surfaces MSLI	More data needed to quantify risk of error
Flow rate	High
Timer operation of solenoid valve (DPI-Testing)	High
Cleanliness of stage nozzles	More data needed to quantify risk of bias
Worn/corroded stage nozzles	More data needed to quantify risk of bias
Electrostatic effects	High, when non-metallic components are used
Use of collection surface coating	High
Environmental factors (barometric pressure, relative humidity)	Potentially high, depending more on formulation e.g. hygroscopic particles than impactor

Only three impactors, the MSLI, ACI and NGI, appear in both the PhEuro and USP. Both Pharmacopoeias state test procedures for all three impactors for use with both MDIs and DPIs. Nevertheless, the MSLI is restricted to DPIs only in USP (2005).

1.1.16.3.1 SSGI (Single-Stage Glass Impinger).

Because it is a simple, easy to use and assemble, and an inexpensive quality control tool, the SSGI has been retained as Apparatus A in the PhEuro and is particular recommended for routine quality control applications. One advantage is that it is made of glass and therefore not affected by corrosion in the same way as conventional metallic impactors. The SSGI operates on the principle of impingement to categorise the dose emitted into non-respirable and respirable dose. Stage 1 represents the non-respirable fraction and consists of the back of the glass throat and the upper impingement chamber, as shown in Figure 1. 14. The respirable dose is collected in the lower impingement chamber known as Stage 2. Its usage is restricted to the evaluation of nebulisers, MDIs and DPIs

The impinger is calibrated at a flow rate of 60 L/min. The particle cut-off size is 6.4 microns and particles < 6.4 um pass into the lower impingement chamber.

1.1.16.3.2 MSLI (multi-stage liquid impinger)

The MSLI is listed as Apparatus 4 in the USP and as Apparatus C in the PhEuro. It consists of 4 impaction stages and a final filter stage. The MSLI is

used for measuring the aerodynamic size distribution of DPIs, in the USP (2005) and for MDIs, DPIs and nebulisers, in the PhEuro (2007).

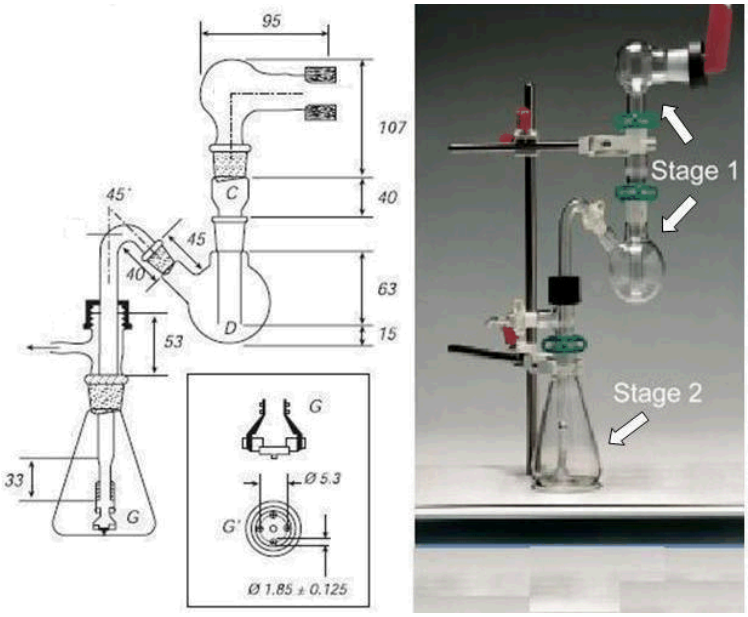

Figure 1.14 Single-stage glass impinger and dimensions in mm tolerances ± 1 mm unless otherwise prescribed. Source: adapted from (Copley, 2007).

The MSLI is manufactured in three different materials aluminium, 316 stainless steel or titanium. One advantage is that it is designed to help reduce the problem of re-entrainment of powder associated with the conventional impactors such as the ACI, NGI and the MMI. This is because the collection stages of the MSLI are kept moist (PhEuro, 2007).

The MSLI requires an induction port to connect it to the inhaler, as shown in Figure 1.15, but does not require a pre-separator to use it with DPIs. Nevertheless, when operating with a DPI, the configuration of the 8-stage impactor specified in the Pharmacopoeia should be followed. The MSLI can be used throughout the range 30-100 L/min

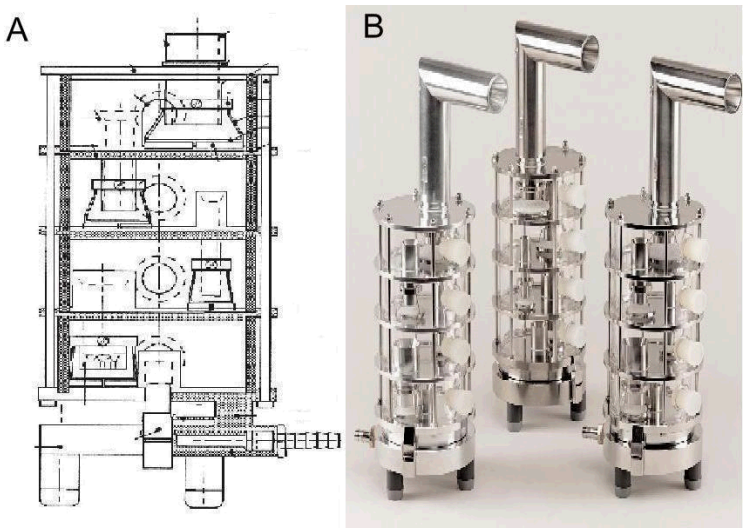

Figure 1.15 A- Schematic of MSLI. B- MSLI (Aluminium, 316 Stainless Steel and Titanium) Source: adapted from (Copley, 2007).

1.1.16.3.3 ACI (Andersen cascade impactor)

The ACI is one of the most commonly used impactors within the pharmaceutical industry for evaluating inhaled products. It was originally developed as a bacteriological air sampler and then adopted by the pharmaceutical industry for characterising size distributions of aerosol products. The ACI is listed as Apparatus 1 for testing MDI products and Apparatus 3 for testing DPI products in the USP and also listed as Apparatus D in the PhEuro.. The ACI consists of eight stages together with a final filter. The stages are clamped together and sealed with O-rings. It can be manufactured from aluminium, 316 stainless steel and also titanium (see Figure 1.16).

Figure 1.16 A- ACI (Aluminium, 316 stainless steel and titanium) and B-Schematic of ACI Source: Adapted from (Copley, 2007).

The ACI has many advantages which may be summarised as follows:

- Well established and accepted by the regulatory authorities.
- Total of 8 individual stages ranging between 0.4 and 9 μm.
- Constructed from three different materials (aluminium, 316 stainless steel or titanium) which give a variety of choice of construction.
- Can be operated at different flow rates, 28.3, 60 and 90 L/min, using a conversion kit for high flow rate testing.
- Low resistance at high flow rates when Stages 6 & 7 are removed.
- Small space needed for operation.
- Stacked design allows damaged stages to be removed and replaced if necessary.

In order to prevent stages overloading, such as in the case of DPI testing, it is necessary to add a preseparator, shown in Figure 1.17, which traps the non-inhalable particles (Mitchell et al., 2003).

Figure 1.17 Pre-separator for Andersen Cascade Impactor. Source: adapted from (Copley, 2007).

1.1.16.3.4 MMI (Marple Miller cascade impactor)

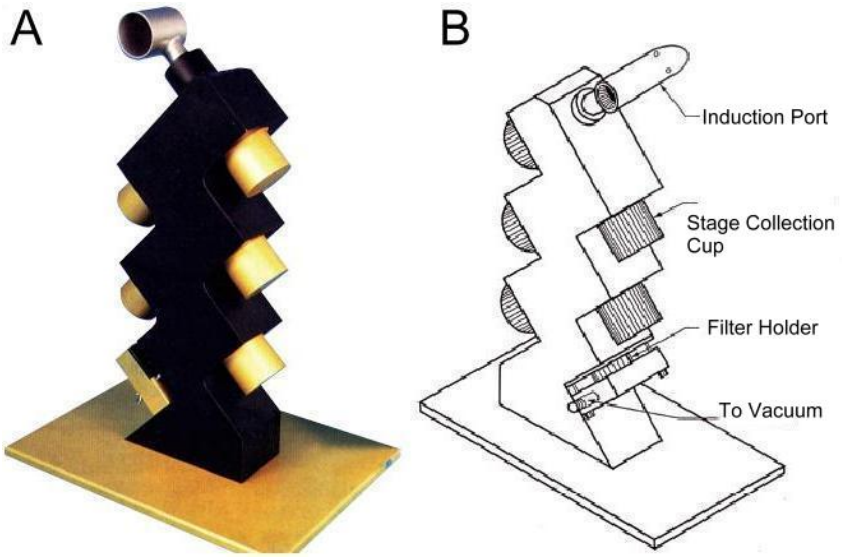

Figure 1.18 A-Marple Miller Impactor, B- Schematic of Marple Miller impactor Source: adapted from (Copley, 2007)

The MMI is listed as Apparatus 2 in the USP, but, is not listed in the PhEuro. MMI is a five-stage cascade impactor used for testing DPIs, as shown in Figure 1.18. MMI consists of five impaction stages and is calibrated to work

in the range 60-90 L/min. Each stage has a detachable collection cup to help in the fast and easy recovery of the drug particles. A paper filter is also placed after Stage 5. It is possible to remove the collection cups after each test without dismantling the impactor. Since the MMI has very low inter-stage losses, it is not necessary to regularly clean the inter-stage passageways between tests (USP, 2005).

1.1.16.3.5 NGI (Next Generation Impactor)

The NGI was released in 2000 and its monographs were later incorporated into the USP as Apparatus 5 for DPIs and Apparatus 6 for MDIs; it is also incorporated in PhEuro as Apparatus E. The NGI was designed specifically for pharmaceutical inhalers. It consists of three main parts: the base frame holding the sampling cup tray, the seal body holding the nozzles and the lid containing the inter-stage passageways, as illustrated in Figure 1.19. It has seven stages and operates in the range between 30 and 100 L/min. The NGI has many areas in which it is better for inhaler testing. One of its features is that particles are collected on cups held in a tray, which can be removed easily, facilitating quick sample handling. Another unique feature is the presence of a micro-orifice collector (MOC) because it captures extremely small particles and in most cases, this eliminates the need for a final filter. Also, as it has low inter-stage losses, the captured particles in the MOC cup can be analysed in the same manner as the particles collected in the other impactor stage cups**.** This aspect is important for automation and particles are more easily dissolved from the collecting surface than from the fibres network in a filter. In addition, the NGI is 50% more productive than the ACI (Marple et al., 2003).

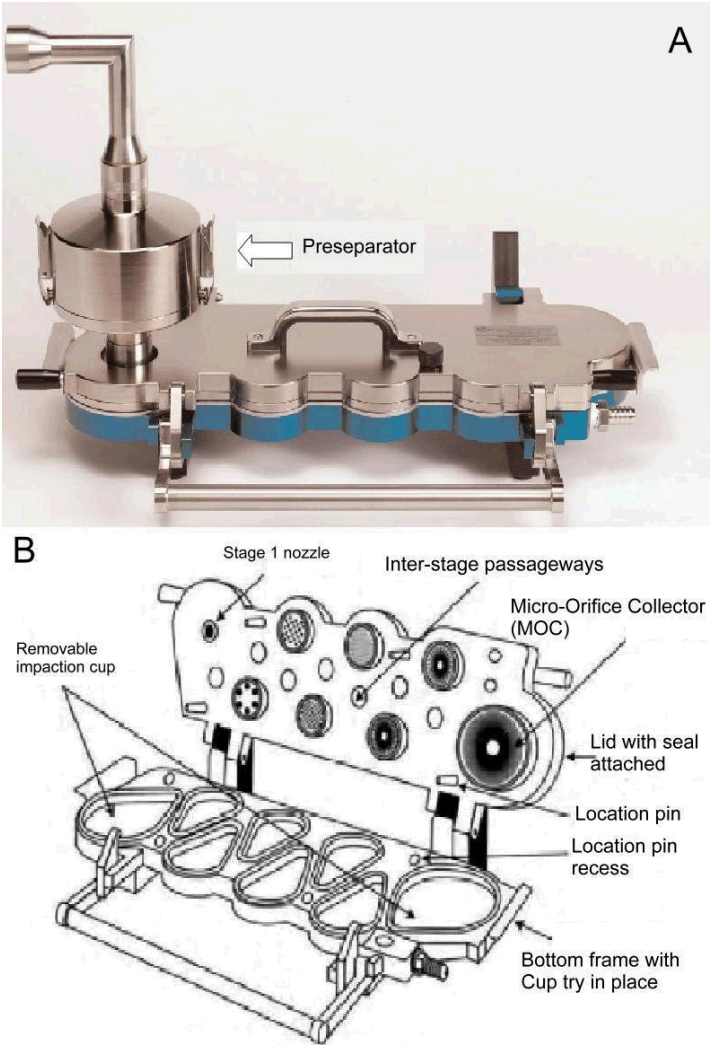

Figure 1.19 A- Next Generation Impactor B- Schematic of Next Generation Impactor Source: adapted from (Copley, 2007, UK-DoH., 2008)

1.1.16.4 Summary

Beyond their value in predicting lung deposition, *in-vitro* studies have an important role in quality control and product development.

Inertial impactors are devices commonly used for the testing of aerosol particles. Their principle of operation is simple: an aerosol stream passes through a nozzle and impinges upon a collection plate. Particles in the aerosol stream with large enough inertia will impact upon the collection plate,

while finer particles with less momentum follow the stream and pass the plate without impacting.

Inertial impactors methods are, however, time consuming. In contrast, optical methods, including TOF analysis, offer rapid measurements, making them attractive. On the other hand, only inertial impactor methods provide direct measure of the mass of API. Furthermore, this technique provides a direct assay of the aerodynamic diameter of most significance in predicting likely deposition in the airways. TOF-analysis also determines aerodynamic size; however, it lacks specificity since it measures the total component of drug rather than API mass.

Finally, it is important to keep in mind that the primary aim of *in-vitro* methods is a relative measure of product performance rather than an absolute measure, since the aim of these methods is to ensure that the product tested is equivalent to the product clinically tested and proven.

PULMONARY DELIVERY DEVICES

1.1.17 Introduction

Inhaled drug products are very popular for drug delivery through the lung or nasal mucosa for local or systemic therapy.

Inhaled bronchodilators and corticosteroids are the mainstay for treatment of asthma and COPD. The inhaled drug devices are classified into three main categories: nebuliser, pMDI or DPI. Most clinical evidence shows that any of these devices will work for most situations and this includes the patient case where the patient is exacerbated or stable (Geller, 2005).

1.1.18 pMDIs (pressurised metered dose inhalers)

1.1.18.1 Introduction

The pMDI was first introduced in the first half of the 20th century and has become the most popular dosage form for the delivery of drug to the respiratory tract. It consist of propellants, drug formulation, a metering valve and actuator, as illustrated in Figure 1.20, all of which play roles in particle size, spray formation and, as a result, in determining drug delivery to the lungs. At first, they were known as metered dose inhaler (MDI), but the term "pMDI" has become more popular, in order to differentiate them from other non-pressurised metered dose devices such as DPIs and other multi-dose devices (Crowder et al., 2001).

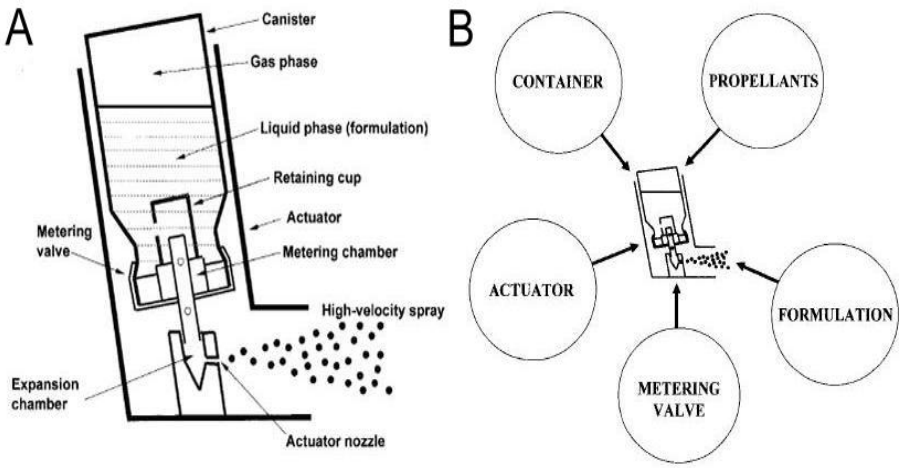

Figure 1.20 A- Schematic of the pMDI. B- The component parts of the pMDI. Source: adapted from (Newman, 2005).

1.1.18.2 Container

Aluminium, stainless steel and glass are used to manufacture the pMDI container or canister. An aluminium canister is preferred, because it is light, robust, light-proof and inexpensive. However, occasionally, due to their

nature glass containers may be preferred for some solution formulations. Usually, the internal surfaces are coated to avoid adhesion of drug particles and chemical degradation of drug. Also, the container can withstand the high pressure generated by the propellants and It must be made of inert materials for drug delivery (Crowder et al., 2001).

1.1.18.3 Propellants

One of the most vital components of a pMDI is its propellant. The propellant creates the force to generate the aerosol cloud. In pMDIs, it is typically liquefied compressed gas, which is in gaseous form at atmospheric pressure, and becomes liquid when compressed. They have to be non-toxic, non-flammable, chemically stable, with constant vapour pressures throughout the product's life, and compatible with drugs.

Chlorofluorocarbons (CFCs) met these requirements and pMDIs have traditionally used CFC as the major propellant. A key property of CFCs was that within a closed canister they formed a 2-phase liquid and saturated vapour system. As a result, a dynamic equilibrium existed between liquid and vapour phases, providing a constant vapour pressure regardless of whether the canister is full or nearly empty (Newman, 2005).

However, pMDIs containing CFCs have recently been replaced because of the effect of CFCs on the ozone layer in the stratosphere and the use of CFCs was banned under international agreement.

As result, hydrofluoroalkanes (HFA) have replaced CFCs. Formulations containing HFAs, either tetrafluoroethane (HFA-134a) or heptafluoropropane (HFA-227), are usually used in formulations. In practice, however, despite the similarities with the CFCs, many challenges in substituting HFAs for CFCs in

pMDIs have been recognised. These include compatibility of the pMDI components, such as valves and container walls, with HFAs.

In addition, it has been found that HFAs are not completely eco-friendly and are greenhouse gases. It is therefore predicted that this may lead to future restrictions on their use, even though their involvement in global warming is expected to be low. As a result, many alternatives have been suggested as propellants, such as dimethyl ether, propane or butane. But propane and butane are likely to be excluded because of their flammability.

Overall, pMDIs which use CFC-free propellants have continued to challenge formulation scientists to develop efficient pMDI devices (Crowder et al., 2001).

1.1.18.4 Drug formulation

Suspensions or solutions are usually used as vehicles for drugs. Since CFCs are non-polar liquids in which many drugs have low solubility and generally good chemical stability, suspensions have often been used in CFC_pMDIs. In order to reduce particle aggregation and lubricate the valve mechanism, surfactants have regularly been used in them. But, with the change to the HFAs system, solubility has become a problem. As a result, co-solvents have been used, such as ethanol, a low-volatility co-solvent in HFA formulations, which initially solubilises surfactants which had previously been approved for use in CFC formulations. More recently, ethanol has been used to solubilise the drug itself in inhaled formulation (Crowder et al., 2001, Newman, 2005).

1.1.18.5 Metering valve

This is incorporated into the container as it is an important component of the pMDI as its main purpose is to ensure the uniformity of the delivered doses. Usually, the metering valve incorporates a metering chamber which holds a single dose, with a volume ranging from 25 uL to 100 uL. In some cases the valve is surrounded by a large reservoir which is able to hold the next dose.

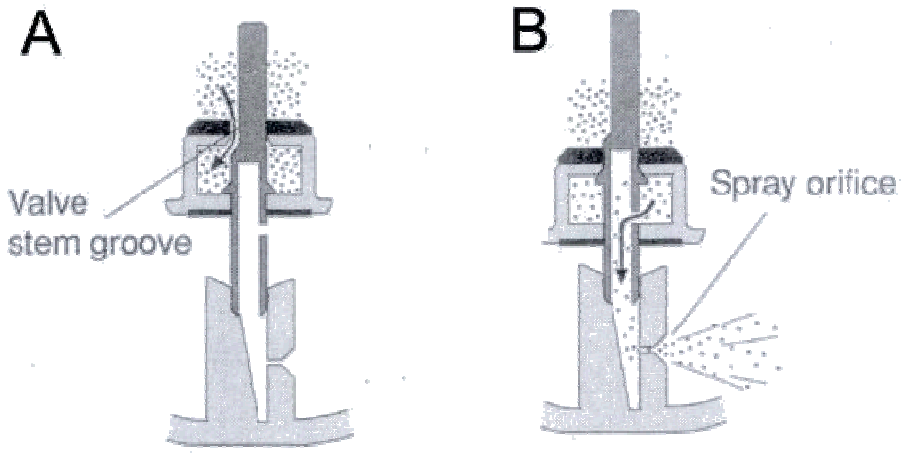

Figure 1.21 A. metering valve in resting state. B. compressed valve Source : adapted from (Bisgaard et al., 2002).

Figure 1.21A shows the metering valve in the resting state; the channel between the body of the canister and the metering tank is opened, as a result the metering tank fills from the canister. As the pMDI is activated by compressing the valve stem, this channel then closes and another channel connecting the metering chamber to the atmosphere opens and the content of the metering tank empties, as shown in Figure 1.21B. When the compression is relieved, the valve retains its position, allowing the metering tank to be refilled (Figure 1.21 A).

Compatibility of other pMDI components, such as propellants, excipients and solvents with the parts of the valve, significantly influences pMDI performance. However, because of solubility in CFCs, elastomeric seals can swell and this has been found to sometimes cause suboptimal operation of the device. In contrast, the seals are less soluble in HFAs, and new elastomeric systems have been developed for use with them. The minimum concentrations of extractable and leachable in the formulation should be borne in mind when the valve elastomers are selected. Many new valves show good performance without the surfactant to lubricate the valve stem (Crowder et al., 2001).

1.1.18.6 Actuator

The actuator is usually made from plastic and its design is a factor in determining the aerosol particle size, particularly the nozzle diameter, which ranges between 0.14 mm and 0.6 mm (Newman, 2005). Its effect on particle size has been studied using gamma scintigraphy, with a formulation containing fenoterol and ipratropium bromide. Newman reported mean lung deposition measured and a step-wise increase from 12.8% of emitted dose to 15.2% 18.0% as a resulted of decreasing the nozzle diameter from 0.3 mm to .25 mm to 0.2 mm. Nevertheless, the 0.2 mm nozzle produced the highest deposition in the mouthpiece. Also, the length of the actuator nozzle path has influence on particle size (Newman et al., 1999).

1.1.18.7 Breath-actuated pMDIs

Patient co-ordination of actuation with inhalation can be a problem with

pMDIs, especially in certain groups of patients such as the young, elderly or chronically ill. One solution is to use breath-actuated pMDIs which may overcome this problem, since they are sensitive to patient inhalation through the device and fire the inhaler simultaneously with their inhalation. The Autohaler, Easibreathe, K-Haler, MD Turbo, Xcelovent, Smartmist are example of such devices and there are several more under development. Another mechanism to overcome this problem is the addition of a spacer device or integrated spacer mouthpiece, and example of these are the Aerohaler, Azmacort pMDI, and Spacehaler (Crowder et al., 2001).

1.1.18.8 Summary

The pMDIs have been used for more than 50 years and are well accepted by most patients as a method of administering inhaled medications. Conventional pMDIs consist of a container, a metering valve, a drug formulation and a propellant. Because of these points, pMDIs provide many advantages for the patients and these are listed in Table 1.3, along with some of the disadvantages.

Table 1.3 Advantages and disadvantages of conventional pMDIs

Advantages	Disadvantages
Convenience, availability to use	Drug delivery significantly affected by inhalation technique
Cost	
Number of doses may reach 100	Propellants needs
High pressure protects contents against bacteria and moisture.	High velocity of particles leads to high oropharyngeal deposition

1.1.19 DPIs (dry powder inhalers)

1.1.19.1 Introduction

The DPIs can be defined as devices through which an active drug is delivered to produce a local or systemic action by the pulmonary route using a dry powder formulation (Islam et al., 2008). At the end of the 1960s, the first DPI was introduced. The major reason was linked to one drug and the need for an increased dose of sodium cromoglycate 20 mg, where the pMDI system was not capable of delivering such a dose, The Spinhaler® (Aventis) was therefore developed to deliver the drug (Chrystyn, 2007).

Successful delivery of drugs deep into the airways relies on the integration between powder formulations and the device performance. Most DPI formulations comprise a micronised drug, mixed with larger carrier particles, which decrease aggregation, improve flow and assist in dispersion. Apart from ability to deliver larger dose, the DPIs have the advantage that they require minimum or no coordination of actuation and inhalation as DPIs are only activated while the patient inhales. Also, because the formulation for DPIs is one-phase, as a solid particle mixture, they are more stable than pMDIs. But the emitted dose depends on the patient's inspiratory airflow (Chrystyn, 2007).

1.1.19.2 Principle of Operation

As indicated above, the majority of DPIs are comprised of micronised drug blended with larger carrier particles; this stops aggregation and improves airflow through the device. When the patient inhales through the DPI, the airflow will create turbulence and shear; the introduction of air into the

powder bed causes the static powder blend to be fluidised to enter the patient's airways. In the airways, the particles of drug separate from the carrier and continue to the smaller airways, while the larger carrier particles deposit in the oropharynx. However, one main disadvantage of DPIs is the low deposition efficiency. This may be partially explained by insufficient drug/carrier separation phased with DPIs, as shown in Figure 1.22. Another concern is dose uniformity.

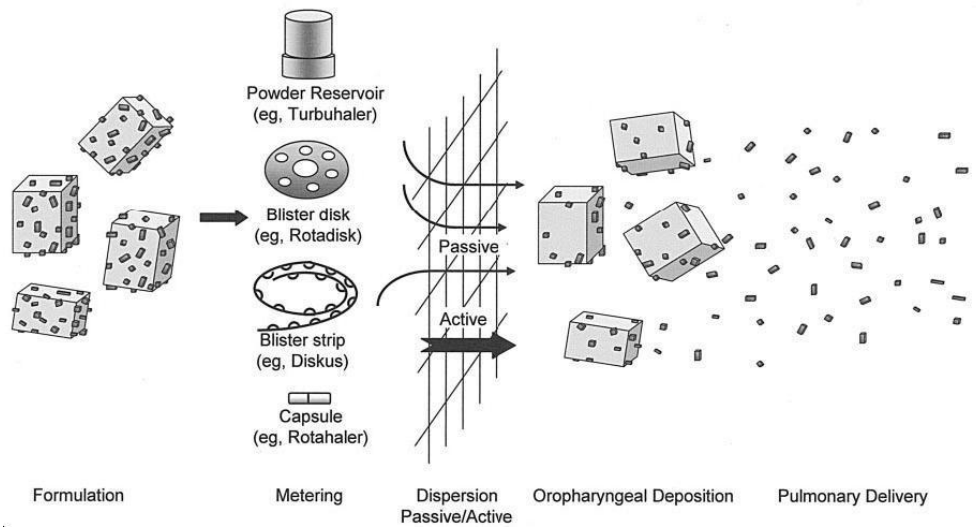

Figure 1.22 Principle of dry powder inhaler design. Source: adapted from (Telko et al., 2005).

A variety of dispersion mechanisms have been used for DPIs. The dominating mechanism is breath-activated, where the aerosol is generated by the patient's inhalation. On the other hand, quite a few power-assisted devices such as vibratory, impact force and pneumatic have recently been developed or are currently under development. In addition, it may be possible to increase the delivery efficiency and reproducibility, if the shear and turbulence could be standardised by using a dispersion mechanism which omits the influence of the patient's breath (Telko et al., 2005).

1.1.19.3 Types of DPI delivery devices

DPI devices are classified by dose type into three categories single-unit dose, multi-unit dose and multi-dose reservoirs. Table 1.4 gives examples of DPIs in the three categories

1.1.19.3.1 Single-unit dose devices

In a single-unit dose device, the drug is supplied in single gelatine capsules, which are then loaded into the inhaler for a single dose and removed after use.

1.1.19.3.2 Multi-unit dose devices

The multi-unit dose device which can hold multiple doses without needing to reload. Each single dose is pre-metered, individually sealed and discharged. Generally, the packaging consists of replaceable disks or cartridges or strips of foil-polymer blister packaging that might or might not be refilled. The multi-unit dose device system has the advantage of the doses being protected from environmental conditions and dose uniformity is enhanced.

1.1.19.3.3 Multi-dose reservoir devices

The multi-dose reservoir device stores the bulk supply of drug and has a built in meter to measure each single dose from the bulk with each actuation. The recently released devices of this type try to overcome the common issues such as decreasing the effect of flow rate dependent on dose emission and the effect of moisture which enters into the device reservoir from the patient during exhalation or from environmental humidity.

Table 1.4 Current DPI devices available in market Source: adapted from (Islam et al., 2008)

Device	DPI type	Delivery method	Drugs	Diseases
First generation: breath-actuated single unit dose				
Spinhaler	SD	Capsule	SC	Asthma
Rotahaler	SD	Capsule	SS BDP SS + BDP	Asthma
Inhalator	SD	Capsule	FN	Asthma
Cyclohaler	SD	Capsule	SS BDP IB BUD	Asthma
Handihaler	SD	Capsule	TT	COPD
Aerolizer	SD	Capsule	FR	Asthma
FlowCaps	SD	Capsule	NA	Asthma
TwinCaps	SD	Capsule	NI	Influenza
Second generation: breath-actuated multi-unit, multiple dose				
Turbohaler	MD	Reservoir	SS TS BUD	Asthma
Diskhaler	MD	Blister package	SX BDP FP ZN	Asthma, Influenza
Diskus/ Accuhaler	MD	Strip pack	SS SX FP SX+ FP	Asthma
Aerohaler	MD	NA	IB	Asthma
Easyhaler	MD	Reservoir	SS BDP	Asthma
Ultrahaler	MD	Reservoir	NA	NA
Pulvinal	MD	Reservoir	SS BDP	Asthma
Novolizer	MD	Reservoir cartridge	BUD	Asthma, COPD
MAGhaler	MD	Reservoir	SS	Asthma
Taifun	MD	Reservoir	SS	Asthma
Eclipse	MD	Capsule	SC	Asthma
Clickhaler	MD	Reservoir	SS BDP	Asthma
Asmanex Twisthaler	MD	Reservoir	MF	Asthma
Third generation: active device				
Exubera	SD	Blister	Insulin	Diabetic
Airmax	MD	Reservoir	FR BUD	Asthma COPD

MF: mometasone furoate, SS: salbutamol sulphate, SX: salmeterol xinafoate, FP: fluticasone propionate, BUD: budesonide, TS: terbutaline sulphate, FN: fenoterol, FR: formoterol, IB: ipratopium bromide, SC: sodium cromoglycate, BDP: beclomethasone dipropinate.NI: Neuraminidase inhibitors, ZN: zanamivir, SC: sodium chromoglycate, TT: Tiotropium, SD: Single dose, MD: Multi-dose

1.1.20 Nebulisers

Nebulisers are devices which can generate aerosol droplets from a liquid and produce a respirable cloud for inhalation (Crowder et al., 2001). Nebulisers fall into two categories: jet nebuliser and ultrasonic nebuliser.

1.1.21 Spacer devices

Due to the high speed of production of the aerosol cloud after actuation, the coordination of actuation and patient inhalation can be very hard. As a result, patient oro-pharyngeal deposition can be high and there is often a big variation in the lung dose. In order to ease the coordination problem and reduce the oro-pharyngeal deposition and lung dose variation, which occurs especially in children and the elderly, spacers and holding chambers have been introduced, as shown in Figure 1.23.

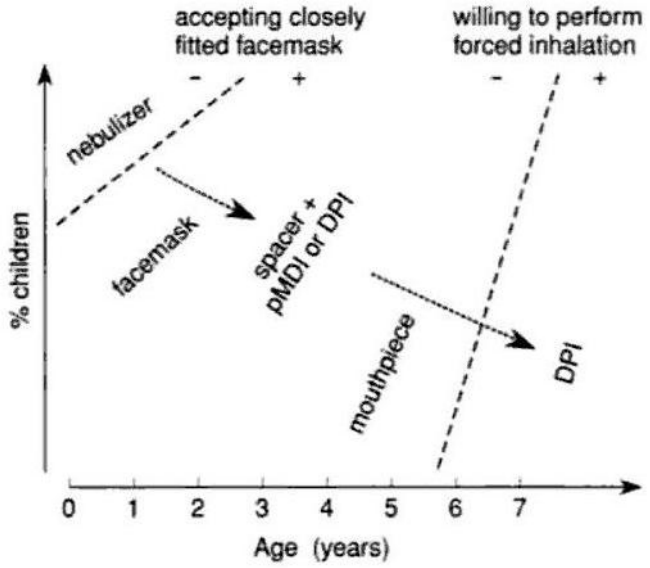

Figure 1.23 Choice of delivery device in children of different ages Source: (Bisgaard et al., 2002).

Spacers were designed to be used with pMDIs, although they have also been used with DPIs (Everard et al., 1996, Matida et al., 2004). The spacer classifies the particles according to their size and large particles are deposited within the spacer rather than in the patient's mouth. This may become very useful in DPIs in which side effects may occur from the deposition of large particles and agglomerates in the mouth, as occurs in pMDIs (Daniher et al., 2008). In clinical practice, the term spacer and holding chamber are often used interchangeably. But the spacers are an extension of the pMDI actuator, allowing the aerosol to decelerate and mature, whereas holding chambers are valved spacers allowing the patient to breathe the cloud of aerosol in the chamber. There are various designs, including large and small volume spacers and tube extensions (see Table 1.5 and Figure 1.25). Especially in young children, it is critical to know the requirement for optimal drug delivery from spacers, because their breathing can be shallow and irregular, although this may be true for many other groups of patients. Over the years, more knowledge and understanding of the technical specification for spacers has developed (Bisgaard et al., 2002).

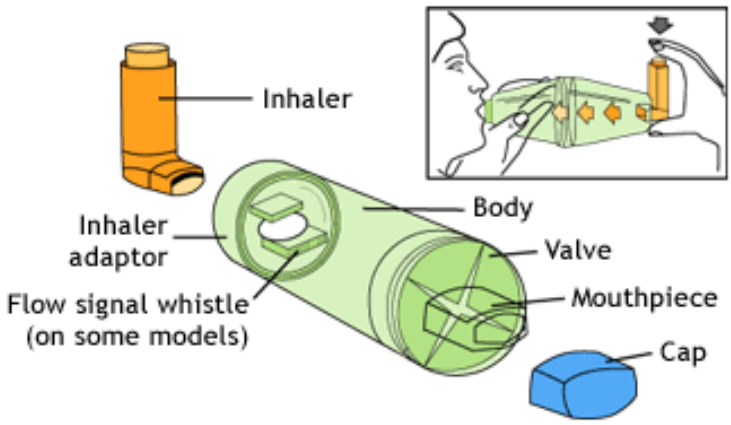

Figure 1.24 Schematic of spacer Source: (ASC, 2008).

Table 1.5 Spacers available in UK source (BNF, 2008)

: Spacer	Description
Able Spacer®	Small-volume device
AeroChamber® Plus	Universal medium-volume device.
Babyhaler®	For paediatric use only. To be used with Flixotide®, Seretide®, Serevent®, and Ventolin® inhalers.
Nebuchamber®	to used with Pulmicort®
Optichamber®	Universal device.
PARI Vortex Spacer®	Universal medium-volume device.
Pocket Chamber®	Universal small-volume device.
Volumatic®	Large-volume device. To be used with Clenil Modulite®, Flixotide®, Seretide®, Serevent®, and Ventolin® inhalers.

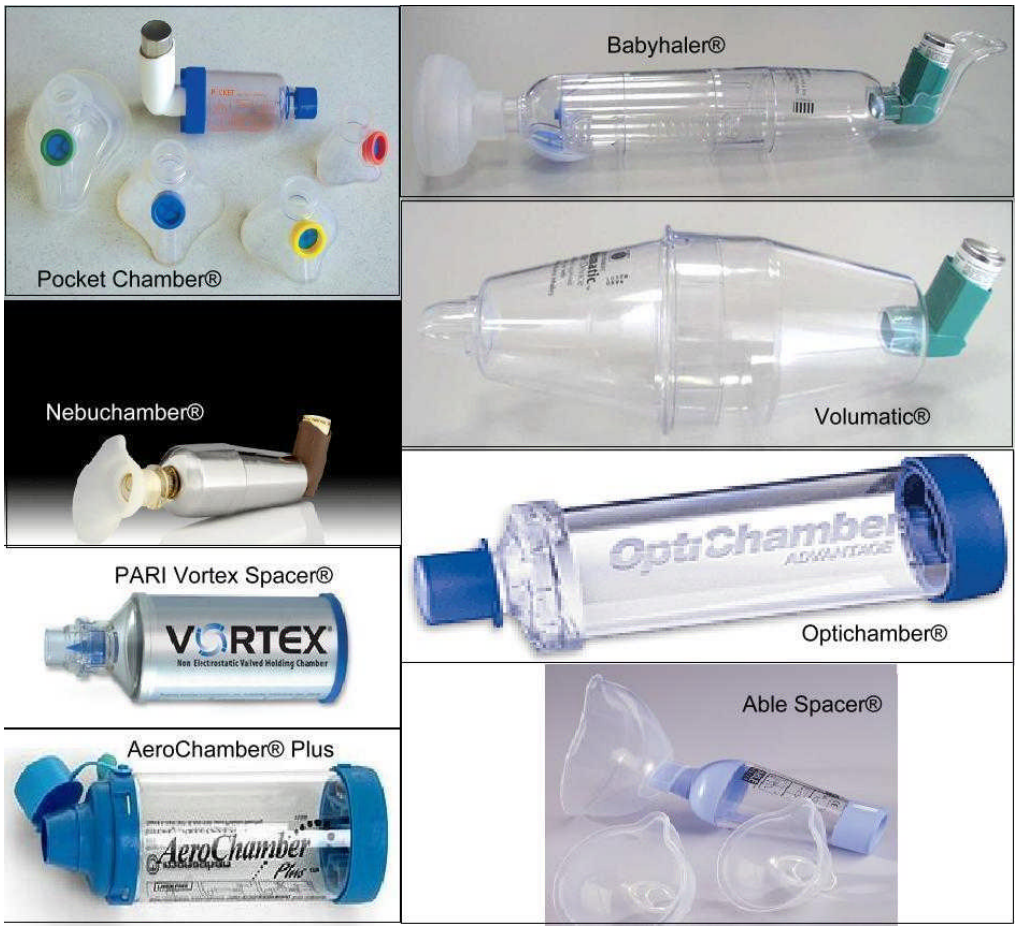

Figure 1.25 Spacers available in UK.

1.1.22 Summary

Aerosol therapy was introduced in the 1950s and became the cornerstone of management of obstructive airway diseases. Large doses can be delivered by nebulisers and small doses by pMDI and DPIs. The main disadvantage of inhaler devices is that they are inefficient as delivery systems to the lung and 2-30% of total emitted dose is delivered, as shown in Table 1.6.

pMDIs continue to be the most popular delivery system because of their safety and efficacy profile in delivering drugs to the airways, but they are associated with high oro-pharyngeal deposition and poor coordination of actuation and inhalation because the emitted dose leaves the canister at high velocity.

However, pMDIs including a spacer have become as effective as nebulisers but are also more convenient for patients to use. The use of spacers may also help to increase the delivered dose to the lung and decrease oro-pharyngeal deposition and eye and skin contact.

But in operation many patients prefer DPI devices to pMDIs because they are easier to use. However, in practice, as DPIs are flow-dependent and in cases where patients have severe airway disease, there can be frequent pulmonary exacerbations or, if used in children, difficulty in achieving suitable flow rate.

A variety of DPI devices are currently available for obstructive disease with an emphasis on optimised drug delivery with low variability. The DPIs also have an advantage of a solid-state phase which makes them more stable products. They also have the advantage of delivery as a single-use disposable unit or multiple-dose refillable systems. A further advantage is

that they are activated by the patient's inspiration and therefore can require a minimum of or no coordination.

Table 1.6 Characteristics of aerosol inhalers. Source: (Khilnani et al., 2008)

	pMDI	DPI	Nebuliser
Technique of generation of aerosol	Propellant based	Patient driven	Bernoulli's principle piezoelectric crystal
Particle size	1-10 µ	1-10 µ	Variable
Drug deposition	5-10%	9-30%	2-10%
Oro-pharyngeal deposition	Significant	Variable	Insignificant
Patient coordination	Required	Not applicable	Not required
Breath hold	Required	Not required	Not required
Patient generation of flow	Not required	Required	Not required
Amount of drug	Small doses	Small doses	Large doses possible
Contamination	No	No	Possible
Use for chronic therapy	Yes	Yes	Rarely
Use for emergency management	No	No	Yes
Use for intubated patients	Preferred	No	Second choice
Cost	Cheap	Cheap	Expensive

pMDI = pressurised metered dose inhaler, DPI = dry powder inhaler

If all these points are taken into account together, in hospital departments / health centres it is often the case that nebulisers are the preferred choice in emergency departments and intensive care units.

As regards the commercially produced inhalers, it is the case that many inhaled drugs may be presented in more than one inhaler system or formulation. So it is important that the health-caregiver is aware that the drug delivery and deposition may be influenced by the drug formulation, the

propellant or the device. It may also be the case that a familiar drug in a new formulation or device may not be equal to the old formulation.

Many reported clinical studies have examined the performance of inhaler devices and concluded that none of the devices shows any clinicaly superiority. Therefore it is suggested that the selection of a device should be guided by a number of other factors, including patient preference, cost and patient age (Telko et al., 2005).

ASTHMA

On of the major uses of inhaled devices is to relieve and treat the asthmatic patients. For this reason, background information is given below to assist in the kind of issues associated with inhalers and disease states associated with drugs at the centre of this thesis.

1.1.23 Introduction

Asthma is defined as a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. This inflammatory disorder is associated with hyper-responsiveness to a variety of stimuli which leads to recurrent episodes of wheezing, breathlessness, chest tightness and cough, particularly at night and in the early morning. These episodes are generally associated with widespread but variable airflow obstruction which is often reversible, either spontaneously or with treatment (Bryne et al., 2006).

1.1.24 Aetiology / Pathophysiology

There are two predominant types of asthma: extrinsic, allergic or atopic asthma and intrinsic or non-allergic asthma (Koda-Kimble, 2008, Helms, 2006).

Several factors may contribute to increase the susceptibility of the development of the disease in liable patients. These include small size at birth, viral infections, diet, exposure to smoking (either passive or active), and environmental pollutants (see Figure 1.26).

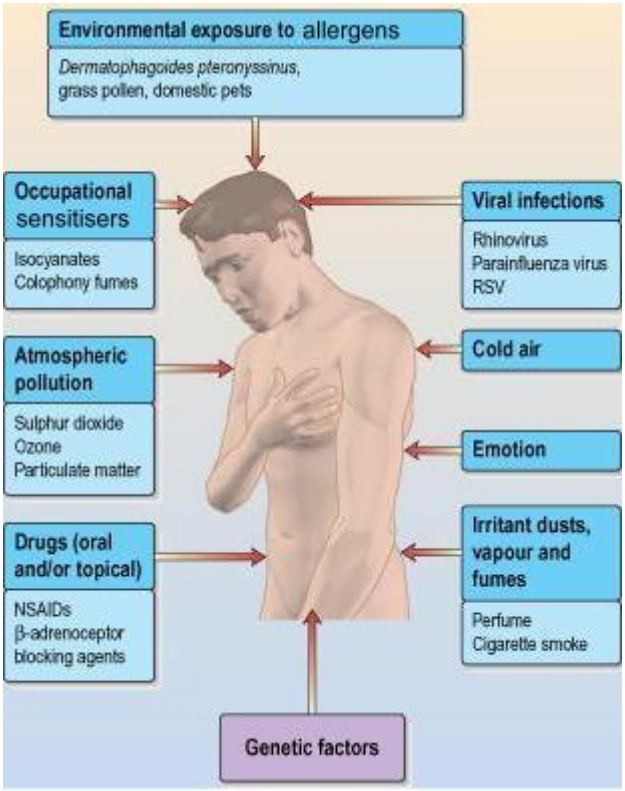

Figure 1.26 Causes and triggers of asthma. RSV (respiratory syncytial virus), NSAIDs, (non-steroidal anti-inflammatory drugs) Source: (Clark et al., 2005).

The pathophysiology of asthma is characterised by a complex interaction between inflammatory cells and mediators. Mast cells, eosinophils, neutrophils, T-lymphocytes and epithelial cells play important roles in the pathophysiology of asthma. Figure 1.27 illustrates the mechanism of cells and mediators associated with airway inflammation.

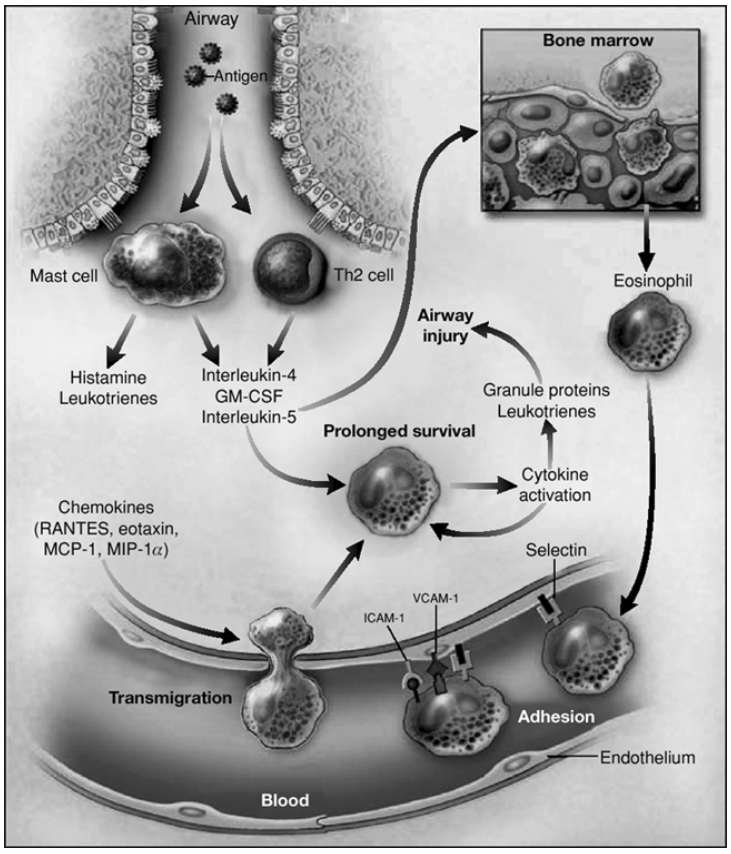

Figure 1.27 Pathophysiologic findings in asthmatic airway. MCP-1, monocyte chemotactic protein; MIP-1, macrophage inflammatory protein; GM-CSF granulocyte-macrophage colony-stimulating factor Source: (Koda-Kimble, 2008).

When an antigen is inhaled the mast cells and TH2 are activated. This leads to release of mediators of inflammation such as leukotrienes and histamine and cytokines, including interleukin-4 and interleukin-5; they later migrate to the bone marrow and enhance final differentiation of eosinophils. Circulating eosinophils in turn moves to the allergic inflammation area. As the eosinophils penetrate the matrix of the airways, their survival is prolonged by interleukin-4 and granulocyte-macrophage colonystimulating factor (GM-CSF).

The eosinophils secrete inflammatory mediators, such as granule proteins and leukotrienes, which cause airways' tissue injuries. Furthermore, eosinophils can secrete GM-CSF to increase their survival and contribute to persistent airway inflammation. However, it should be borne in mind that over 20 cytokines have been reported that could be involved in the inflammation process. Therefore, it is not surprising that drugs which target the presence of one cytokine have not been successful in treating asthma (Koda-Kimble, 2008, Helms, 2006).

More recently, remodelling of the airways is thought to be secondary of failure to control airway inflammation in asthmatic patients. Airway remodelling (Figure1.28) refers to structural changes causing irreversible narrowing of the airway lumen and airflow obstruction which can eventually decrease lung function (Helms, 2006).

Figure 1.28 Remodelling of airways of asthmatic patients

1.1.24.1 Prevalence of asthma

Asthma varies from 1% to 18% in the population in different countries (Figure 1.29).

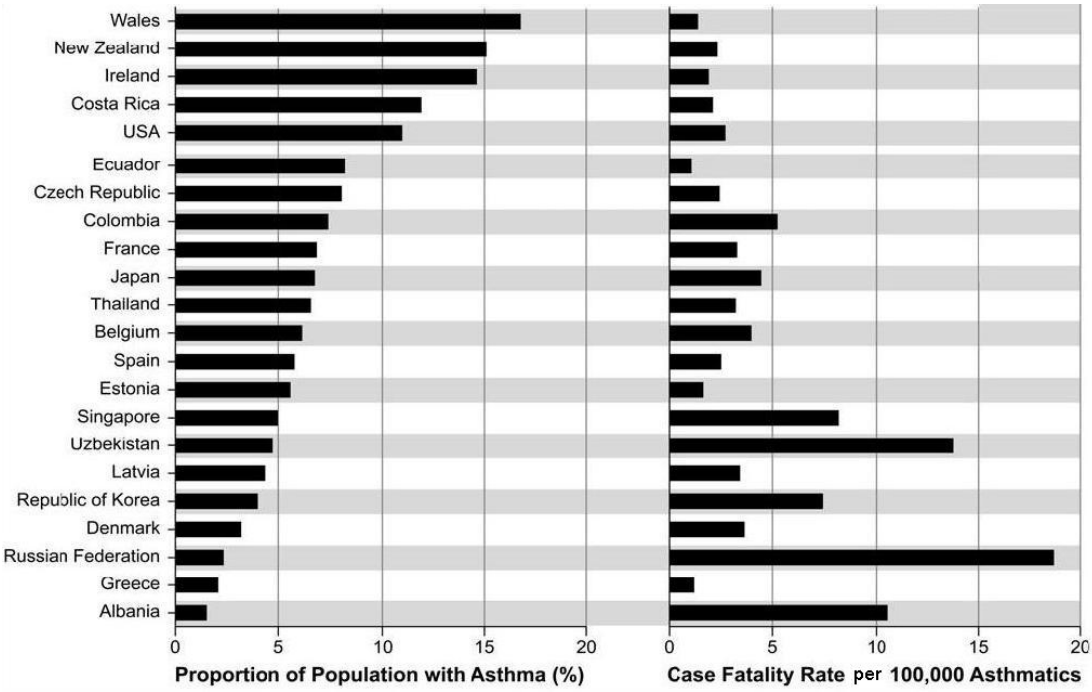

Figure 1.29 Asthma prevalence and mortality. Source: adapted from (Bryne et al., 2006).

1.1.25 Symptoms

Usually, patients with asthma present with symptoms such as cough, episodic breathlessness, wheezing, cough, and chest tightness (Bryne et al., 2006).

1.1.26 Diagnosis

An accurate diagnosis of asthma is essential for appropriate treatment to be started. However, the absence of a gold standard definition leads to making

clear evidence-based recommendations on a diagnosis of asthma not possible.

1.1.26.1 History

The diagnosis of asthma is based primarily on taking a comprehensive history of the symptoms (DiPiro et al., 2008).

1.1.26.2 Pulmonary function tests

1.1.26.2.1 Spirometry

The normal value for the forced expiratory volume in one second (FEV1) forced vital capacity (FVC), FEV1 / FVC ratio in healthy persons is generally 75% to 80%. Age, gender, height, weight and race influence the lung volume The patient's spirometry result is compared with a predicted normal values table for patients with similar physiological characteristics (BTS, 2008).

1.1.27 Asthma classification

Classification of asthma severity is important to establish the treatment plan. Patients are classified into three age groups, 0-4, 5-11 and >12 years, Expert Panel Report 3 (EPR-3) uses the following classifications of intermittent, mild persistent, moderate persistent and severe persistent asthma. Using the frequency of symptoms as essential components of asthma classification (Koda-Kimble, 2008) (see Appendix B).

1.1.28 Non-pharmacological management

As mentioned before, there are various dietary, environmental and other factors which trigger asthma. Avoiding these triggers may help in reducing the need for pharmacotherapy (BTS, 2008).

There is also a major concern regarding asthmatic patients increasing use of alternative and complementary therapies such as herbs, vitamins, massage, black tea, coffee, ephedra, marijuana, dried ivy leaf extract, acupuncture, meditation, homeopathy and yoga. As there are no established clinical studies to support the use of these alternatives there is considerable concern over the expanding use of these methods (BTS, 2008, Koda-Kimble, 2008).

In addition, other strategies have been tried, including smoking cessation and weight reduction (BTS, 2008).

1.1.29 Pharmacological management

1.1.29.1 Goals of therapy

The major goal of asthma therapy is to control the disease. The key of this control are:

- No daytime symptoms
- No night-time awakening due to asthma
- No need for reliever medication
- No exacerbations
- No limitations on activity, including exercise
- Normal lung function (in practical terms, FEV1 and/or Peak expiratory flow (PEF) >80% predicted or best).
- Minimal side effects.

In practice, patients may have different aims and wish to balance the goals of asthma therapy and the possible side effects or inconvenience of taking medication required (BTS, 2008). Table 1.7 shows the level of asthma control.

A stepwise approach aims to control asthma as soon as possible and, to achieve this, patients should begin management at the step closest to the initial severity of their asthma. To maintain the control, stepping-up or stepping-down of treatment is often carried out to achieve control (BTS, 2008) (see Appendix B).

Table 1.7 Levels of asthma control Source: (Bryne et al., 2006)

Characteristic	Controlled (All of the following)	Partly Controlled (Any measure present in any week)	Uncontrolled
Daytime symptoms	None (twice or less/week)	More than twice/week	Three or more features of partly controlled asthma present in any week
Limitations of activities	None	Any	
Nocturnal symptoms /awakening	None	Any	
Need for reliever/ rescue treatment	None (twice or less/week)	More than twice/week	
Lung function (PEF or FEV_1)**	Normal	< 80% predicted or personal best (if known)	
Exacerbations	None	One or more/year*	One in any week***

* *Any exacerbation should prompt review of maintenance treatment to ensure adequacy.*
** *Lung function is not a reliable test for children 5 years and younger.*
***By definition, an exacerbation in any week makes that an uncontrolled asthma week.*

COPD

1.1.30 Introduction

Chronic obstructive pulmonary disease (COPD) is characterised by airflow obstruction that is progressive and not fully reversible. It takes several months to change significantly. The main cause is smoking (NICE-Guideline,

2004a).The two main forms of COPD are chronic bronchitis and emphysema (Helms, 2006).

1.1.31 Epidemiology and aetiology

Long-term exposure to toxic gases and particles can cause COPD. In particular, cigarette smoke, which accounts for over 90% of cases of COPD. Fortunately, only 10-20% of heavy smokers develop COPD.

It is also the case that the incidence of COPD is correlated to the number of cigarettes smoked per day; furthermore, the death risk from COPD in patients who smoke 30 cigarettes per day is 20 times more than for a non-smoker (Figure 1.30).

Figure 1.30 Influence of smoking on airflow limitation Source: (Clark et al., 2005).

The number of patients diagnosed with COPD has been falling steadily; also, the death rate has fallen in the previous 25 years from 200 to 70 per 100,000 (Figure 1.31). In addition, it is the forecast that COPD will become the third most common cause of death and the fifth most common cause of disability worldwide by 2020, as shown in Table 1.8.

Table 1.8 Changes in ranking for most important causes of death from 1990 to 2020 in baseline scenario Source: (Murray et al., 1997)

Disorder	Ranking		Change in ranking
	1990	2020 baseline model	
Within top 15			
Ischaemic heart disease	1	1	0
Cerebrovascular disease	2	2	0
Lower respiratory infections	3	4	-1
Diarrhoeal diseases.	4	11	-7
Perinatal disorders.	5	16	-11
Chronic obstructive pulmonary disease. (COPD)	6	3	+3
Tuberculosis	7	7	0
Measles	8	27	-19
Road-traffic accidents	9	6	+3
Trachea bronchus and lung cancers	10	5	+5
Malaria	11	29	-18
Self-inflicted injuries	12	10	+2
Cirrhosis of liver	13	12	+1
Stomach cancer	14	8	+6
Diabetes mellitus	15	19	-4
Outside top 15			
Violence	16	14	+2
War injuries	20	15	+5
Liver cancer	21	13	+8
HIV	30	9	+21

1.1.32 Pathophysiology

Pathological changes are hypertrophy and increase in the number of mucus-secreting goblet cells of the bronchial tree, consistently distributed throughout the lung but mainly in the larger bronchi. In advanced cases, the bronchi are significantly inflamed and pus can be seen in the lumen (Figure 1.32).

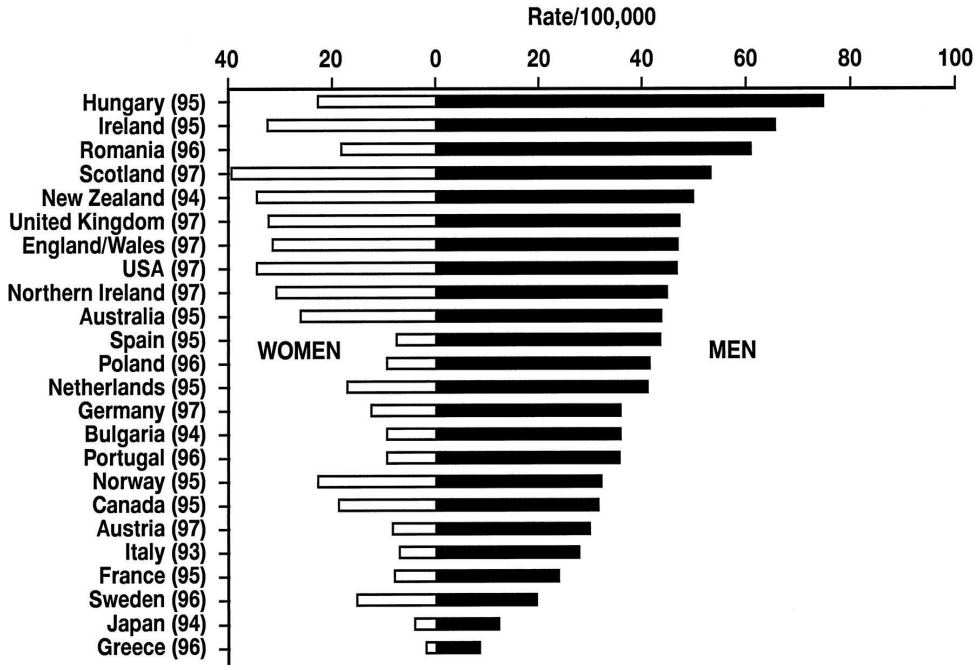

Figure 1.31 Age-adjusted death rates for COPD by country and sex, ages 35 to 74 Source: adapted from (Hurd, 2000).

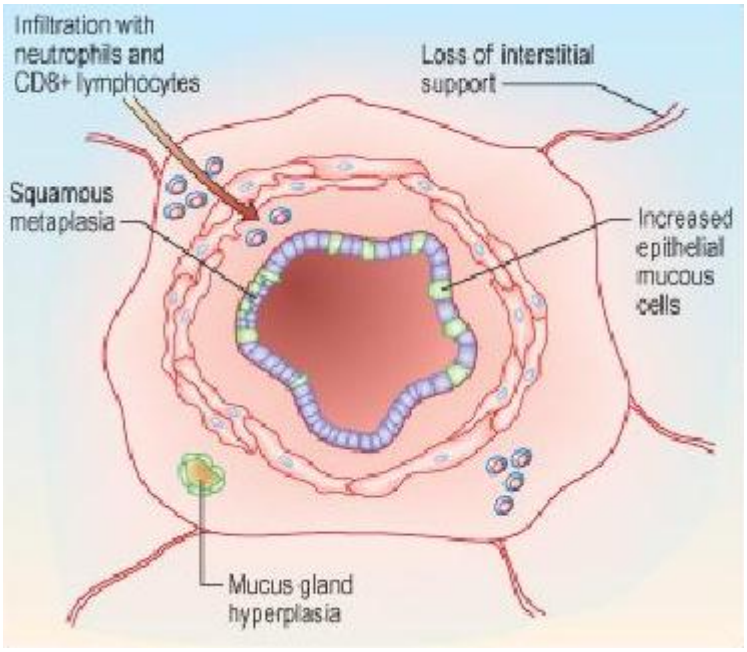

Figure 1.32 Pathological changes in airways in chronic bronchitis and emphysema. Source: (Clark et al., 2005).

Cigarette smoking plays an important role in the pathology of the COPD through the presence of neutrophils in the lumen of the bronchial tree. Additionally, granulocytes infiltrate the small airways of smokers. The granulocytes release elastases and proteases, which may enhance the development of emphysema. α1-Antitrypsin inhibitor is an antiproteinase inhibitor produced in the liver, which then it travels into the blood and penetrates the lung. Its functions are to neutralise neutrophils elastase and α1-Antitrypsin is a main serum antiprotease which may be inactivated by cigarette smoke. Furthermore, hypertrophy of mucous glands in the larger airways may result from repeated irritation from the inhalation of cigarette smoke. Also, the smoke has a bad impact on lung surfactant (Clark et al., 2005).

1.1.33 Diagnosing COPD

The diagnosis of COPD depends on the suspicion as the cause of breathlessness or cough. The diagnosis is based on symptoms and signs and supported by spirometry (NICE-Guideline, 2004f).

1.1.33.1 Symptoms

At an early stage, they are not obvious or disappear and, as the COPD progresses, the symptoms vary among individuals (NICE-Guideline, 2004f). They include productive cough, wheezing and breathlessness. Often, the patient has been a smoker for a long time. As the disease develops severe breathlessness may occur even after mild effort such as dressing (Clark et al., 2005).

1.1.33.2 Signs

In the early stage the signs are limited to wheezing throughout the chest. In severe disease, they include the patient's being tachypnoeic, with prolonged expiration, use of accessory muscles, hyperinflated chest, wheeze or quiet breath sounds, pursed lip breathing, cyanosis, raised jugular venous pressure (JVP) and/or cachexia (NICE-Guideline, 2004f).

1.1.34 Assessment of severity

The assessment of severity is essential because it has implications for treatment and relates to prognosis. One of the tools of classification is spirometry, which can be used to assess the severity of airflow obstruction and to guide treatment and predict prognosis (Rabe et al., 2007).

1.1.35 Complications

During the later stages of COPD, the patient may develop respiratory failure. Also, at this stage, chronic hypoxemia and hypercapnia can cause persistent vasoconstriction in the lung vascular bed, particularly the small pulmonary arteries, subsequently pulmonary arterial hypertension. COPD patients may develop cor pulmonale at the advanced stage, defined as an alteration in the structure and function of the right ventricle secondary to disorder of the respiratory system. It is characterised by right ventricular hypertrophy, pulmonary hypertension and, finally, right heart failure (Helms, 2006).

1.1.36 Managing stable COPD

COPD is a heterogeneous disease, and the therapy plan should be individualised, based on symptoms and disability of the targeted patient (NICE-Guideline, 2004g).

1.1.36.1 Smoking cessation

A smoking history should be documented and patients should be encouraged to stop (NICE-Guideline, 2004g).

1.1.36.2 Inhaled bronchodilator therapy

COPD is characterised by considerably irreversible airflow obstruction; however, bronchodilators have been the central component of pharmacotherapy (Figure 1.33). β2-agonists, anticholinergics and theophylline have all been used in COPD. Beside their direct bronchodilation, both β2-agonists and anticholinergics also reduce static and dynamic hyperinflation (NICE-Guideline, 2004g).

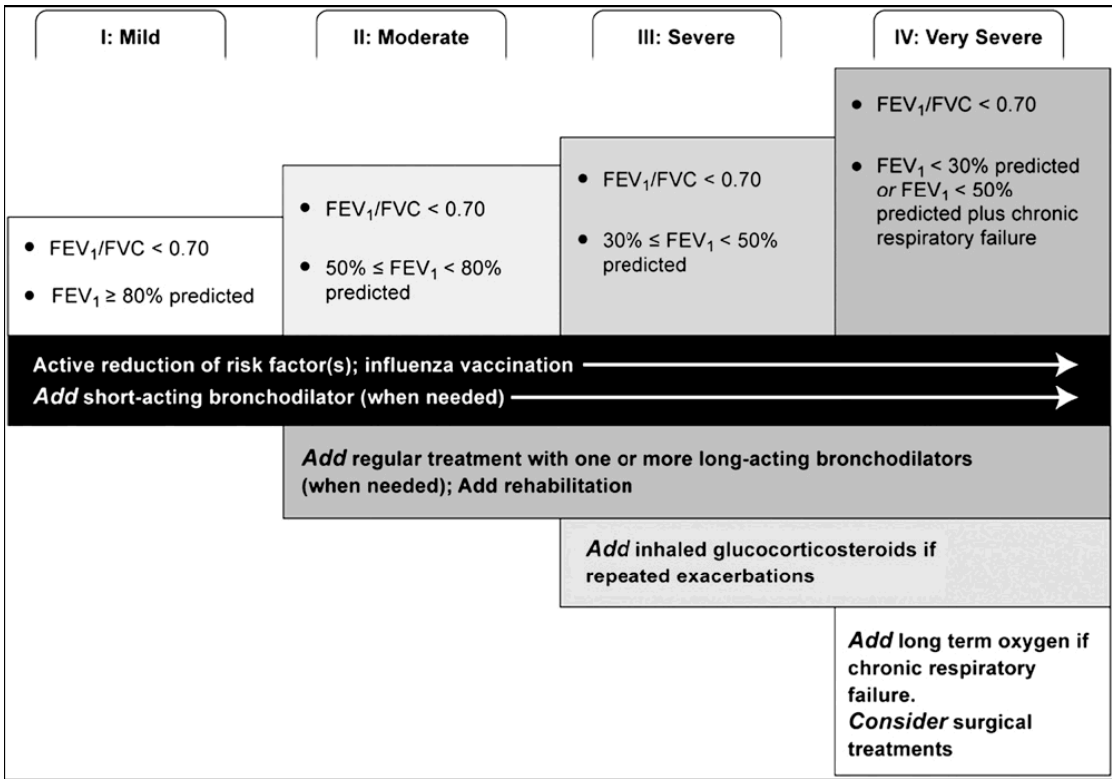

Figure 1.33 Therapy at each stage of COPD. Post-bronchodilator FEV1 is recommended for diagnosis and assessment of the severity of COPD. Source: (Rabe et al., 2007)

1.1.36.3 Theophylline

Theophylline has many advantages, such as an increase in diaphragmatic strength in COPD patients, an effect on mucociliary clearance, anti-inflammatory effects and extra-pulmonary effects, mainly enhancement in cardiac output. But it has a potential toxicity and marked interactions with other drugs. As a result, theophylline is kept for patients who do not tolerate or fail to respond adequately to a combination of inhaled bronchodilators or in patients who are unable to use inhaled therapy. However, because of its different pharmacokinetic profile and interaction with other medications, it should be cautiously used in the elderly (NICE-Guideline, 2004g, Koda-Kimble, 2008).

1.1.36.4 Corticosteroids

Unlike eosinophils, neutrophils are less sensitive to steroids, even if a high dose of inhaled steroid is used. Currently, not all of the inhaled corticosteroids are licensed to be used alone for therapy of COPD patients. Inhaled corticosteroids should be considered for patients with an FEV1 < 50% predicted, who are having ≥ 2 exacerbations per year, which urges treatment with antimicrobials or systemic corticosteroids. The goal of therapy is to reduce exacerbation frequency and decrease the decline in health status and not to provide an improvement in lung function (NICE-Guideline, 2004g).

1.1.36.5 Combination therapy

Bronchodilators work through different mechanisms, hence combining drugs of these classes may add some clinical benefits to patients. This approach

may provide an additional advantage of reduction of the potential side effects of the drugs by avoiding having to use individual drugs near to their maximum dose. In addition, the combination of bronchodilator with an inhaled steroid may produce additive or synergistic clinical benefits (NICE-Guideline, 2004g).

1.1.36.6 Supplemental Oxygen Therapy

In the advanced stage of COPD, patients frequently become hypoxaemic. Many patients develop a tolerance for mild hypoxaemia; however, once the PaO2 falls below 8 kPa, patients start to develop signs of cor pulmonale, mainly peripheral oedema. Oxygen is used to improve exercise capacity and decrease disability in these patients. In addition, oxygen is used to relieve the breathlessness symptom (NICE-Guideline, 2004g).

1.1.36.7 α-1 antitrypsin replacement therapy

α-1 antitrypsin deficiency accounts for around 2% of cases of COPD Recombinant α-1 antitrypsin is currently available and replacement therapy has been proposed as a method of treating patients with α-1 antitrypsin deficiency. However, the national clinical guideline on management of COPD in adults in primary and secondary care does not recommend α-1 antitrypsin replacement therapy in the management of patients with α-1 antitrypsin deficiency (NICE-Guideline, 2004g).

1.1.36.8 Anti-oxidant therapy

There is currently good evidence linking the oxidative stress and severity of disease. The national clinical guideline on management of COPD in adults in

primary and secondary care does not recommend use of vitamin E and beta-carotene supplements, alone or in combination (NICE-Guideline, 2004g).

1.1.36.9 Mucolytic therapy

Mucolytic agents should be considered in patients with a chronic productive cough. Some of these drugs, particularly N-acetylcysteine, may also have antioxidant effects which may contribute to their clinical effects. (NICE-Guideline, 2004g).

ASTHMA AND COPD: DIFFERENCES AND SIMILARITIES

Asthma and COPD disorders cause obstructed airflow and the symptoms of cough, wheeze and breathlessness; moreover, both illnesses can coexist in the same patient. However, despite sharing some clinical features, they differ significantly in aetiology, pathology and management (Koda-Kimble, 2008).

Asthma is usually completely reversible. Patients with asthma respond well to anti-inflammatory medication, including inhaled corticoids. In addition, unless an acute exacerbation is existing, significant gas exchange abnormalities are rare. COPD, on the other hand, is a progressive and often fatal disease. Although bronchodilators are useful in COPD, the degree of bronchodilator reversibility is clearly less than in asthma. Furthermore, the benefits of anti-inflammatory drugs, including inhaled corticoids, are much lower than in COPD.

Patients with COPD, especially those with emphysema, have considerable derangements in pulmonary gas exchange, even at baseline. They generally have a chronic cough, typically productive, and varying degrees of exertional

dyspnoea. Tables 1.9 and 1.10 illustrate the difference between asthma and COPD.

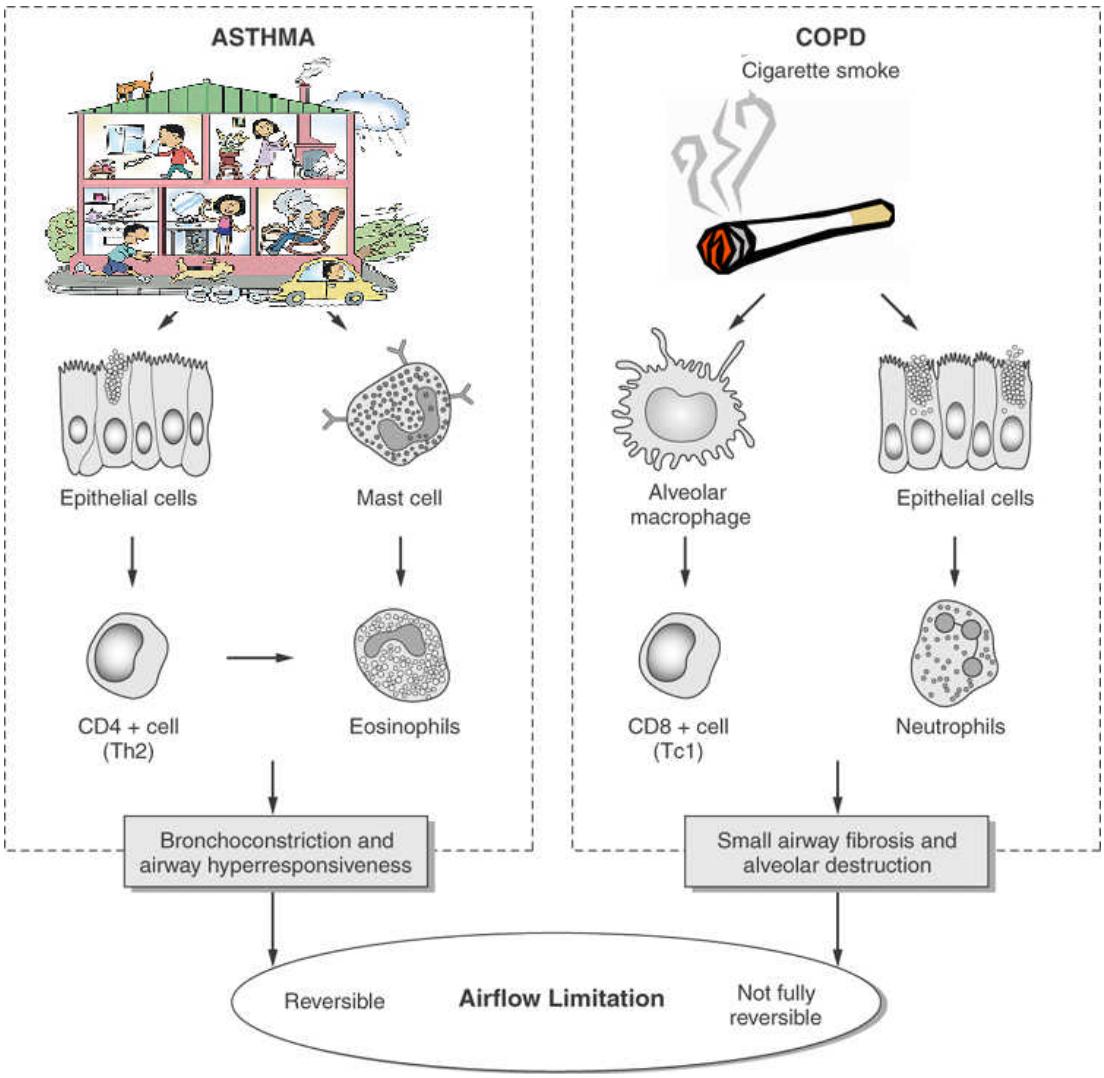

Figure 1.34 Inflammatory cascade in COPD and Asthma. Source: adapted from (Rabe et al., 2007).

Table 1.9 Differences in pulmonary inflammation between asthma and COPD Source: (Rabe et al., 2007)

	COPD	*Asthma*
Cells	Neutrophils ++ Macrophages +++ CD8+ T cells (Tc1)	Eosinophils ++ Macrophages + CD4+ T cells (TH2)
Key Mediators	IL-8, TNF-α, IL-1β, IL-6, NO+	Eotaxin,IL-4, IL-5, IL-13, NO+++
Oxidative stress	+++	+
Site of disease	Peripheral airways Lung parenchyma Pulmonary vessels	Proximal airways
Consequences	Squamous metaplasia Mucous metaplasia Small airway fibrosis Parenchymal destruction Pulmonary vascular remodeling	Fragile epithelium, Mucous metaplasia ↑ Basement membrane Bronchoconstriction
Response to therapy	Small bronchodilator response Poor response to steroids	Large bronchodilator response Good response to steroids

NO= nitric oxide, TNF= Tumour necrosis factor, IL= Interleukin

1.1.37 Managing patients with mixed asthma and COPD

Some patients may show lung function characteristics of asthma and of COPD and the safest option is to treat them as having asthma but to expect COPD outcomes.

Under-estimating the asthmatic component of mixed lung disease exposes the patient to being left without inhaled steroids and other preventive therapy with possibility of fatal consequences. Overestimating the asthmatic component may cause an overtreatment of the patient and perhaps denial of access to respiratory rehabilitation and to less use of relievers (Crockett, 2003).

Table 1.10 asthma versus COPD Source: (Helms, 2006).

Feature	**Asthma**	**COPD**
Past or current history of cigarette smoking	Maybe	Usually
Symptoms present before age 40 years	Common	Rare
Spirometry improvement after bronchodilator	≥12%	Minimal
Chronic productive cough present	Uncommon	Common
Breathlessness	Intermittent	Persistent
Night-time awakening with dyspnoea /wheeze	Common	Uncommon
Significant intra- and inter- day variability in symptoms	Common	Uncommon

ROLE OF PHARMACIST IN IMPROVING ASTHMA AND COPD PATIENT CARE

Whether they work in community pharmacies, hospitals or other health settings, pharmacists are in an essential position to contribute to overall management of patients with asthma and COPD and can educate patients by providing information on the type and purposes of their medication and by demonstrating how to use inhaled drugs and peak flow meters. Also pharmacists can be a valuable source of drug information for all members of the health care team by monitoring medication use and refill periods and use this information to alert prescribers and help identify poorly controlled patients.

The visits to the emergency department and the patient's admission to hospital are the largest portion of the total cost of illnesses, which can be reduced by better control of disease (Lenfant, 1995).

DRUGS STUDIED

This thesis is based around the examination of three mainstream inhaled drugs, formoterol, budesonide and beclomethasone, for the treatment of asthma and COPD.

1.1.38 Budesonide

Budesonide is a corticosteroid, designated chemically as (RS)-11ß,16α,17,21 -Tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with butyraldehyde. Budesonide is provided as a mixture of two epimers 22R and 22S. The empirical formula of budesonide is $C_{25}H_{34}O_6$ (Figure1.35) and its molecular weight is 430.5 (Martindale et al., 2006).

It is a glucocorticoid with a high ratio of local to systemic anti-inflammatory activity due to the absence of halogen atoms on the corticosteroid nucleus, which contributes to the optimal topical-to-systemic activity ratio of budesonide.

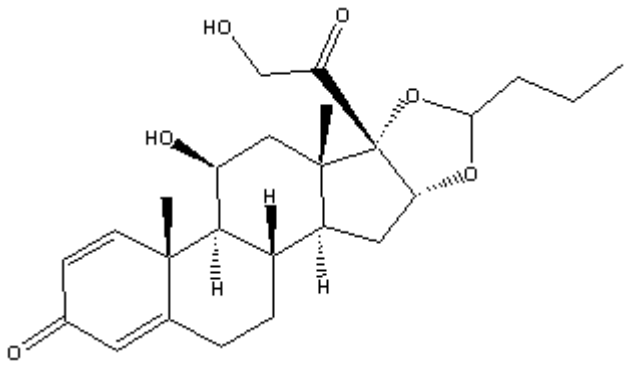

Figure 1.35 Budesonide structure

1.1.38.1 Physical properties

Budesonide is a white to off-white, tasteless, odourless powder practically insoluble in water and in heptane, sparingly soluble in ethanol and freely

soluble in chloroform (UK-DoH., 2008). Its partition coefficient between octanol and water at pH 7.4 is 1.6 x 10^3.

Its high lipophilicity is an advantageous property associated with a high receptor affinity.

1.1.38.2 Pharmacokinetics

The highest concentration (t_{max}) is reached after 30 minutes of oral powder inhalation after administration of budesonide at 400 μg, 800 μg or 1600 μg twice daily via the Turbuhaler which results in a highest concentration (C_{max}) of 1.43 nmol/L, 2.55 nmol/L, and 5.37 nmol/L after the first dose, respectively (SYMBICORT®_Product_Information, 2004). Work by Kaiser and co-workers (1999) have shown that administration of the above doses for 3 weeks resulted in a C_{max} of 2.03 nmol/L, 3.64 nmol/L and 5.37 nmol/L, respectively. In these studies, the t_{max} was achieved at between 10 and 20 minutes for all doses except single-dose budesonide 800 μg. When an oral suspension inhalation was used, the t_{max} was reached after 10 to 30 minutes (Szefler, 1999).

The oral bioavailability from the oral dosage form (capsule) is about 9% after a single dose and around 11% after repeated dosing (Klasco, 2005a).

Budesonide is known to undergo extensive first pass metabolism of around 85% and metabolises to give two inactive metabolites, 16α-hydroxyprednisolone (24%) and 6-β-hydroxybudesonide (5%) (Brunton et al., 2006). For oral inhalation, the bioavailability was 73% (Ryrfeldt et al., 1982).

The total protein binding is 85% to 90% (Klasco, 2005a), with a volume of distribution (Vd) of 3 L/kg(Klasco, 2005a).

Budesonide is then excreted mainly through renal excretion (60%) as metabolites and not as unchanged drug (Ryrfeldt et al., 1982).

Budesonide has a half-life for the parent drug of around 2 to 3 hours. Furthermore, esterification can lead to increases in the retention time of budesonide in the lung. It has been shown that lung deposition of budesonide is twice as high as when administered by DPI versus pMDI, because it produces a higher proportion of fine particles compared to other devices (O'Connell, 2003).

The local adverse effects are lower when a DPI budesonide is used, rather than a pMDI, and the reported incidences are 5.8% vs 17% (O'Connell, 2003).

1.1.38.3 Monitoring parameters

The important parameters of drugs are therapeutic value and toxicity.

1.1.38.3.1 Therapeutic

An initial study often completed is a pulmonary function test, especially peak expiratory flow (PEF) (DiPiro, 2005). Secondly, physical examination is regularly carried out, such as decreased wheezing, dyspnoea and respiratory rate, and decreased number of exercise-induced asthma attacks. (DiPiro, 2005).

1.1.38.3.2 Toxicity

The toxic effect of budesonide can be reduced in the following way. For patients suspected of over-using inhaled corticosteroids or on chronic systemic, the hypothalamic-pituitary-adrenal axis should be monitored by the adrenocorticotropin stimulation test, morning plasma cortisol levels and

urinary free cortisol test. It may then be seen that blood glucose, cholesterol, triglycerides and low-density lipoproteins may be elevated in patients treated with corticosteroids (Klasco, 2005b).

Secondly physical examinations should be carried out for common toxicity from corticosteroids, initially the endocrinal effect which is the most common effect as the Cushing syndrome. Also, the effect on growth of children can be monitored by measuring the height velocity every 3 to 6 months .

Children should be screened annually for any ocular toxicity. Additionally, a common adverse effect for inhaled corticosteroid is oropharyngeal candidiasis. The signs and symptoms are the appearance of white, milky plaques on the tongue which can be avoided by instructing patients to wash their mouths after inhaler use. As regards the effects of corticosteroid on bone, including osteoporosis (Ledford et al., 1998).

1.1.38.4 Place in therapy

Current treatment guidelines, such as *The British Guidelines on Asthma Management* (BTS, 2008), emphasise the use of inhaled corticosteroids (ICS) as first-line therapy for managing persistent asthma symptoms in both children and adults. Compared to 'as needed use' of β-agonists alone, ICs generally have been shown to increase FEV1, decrease airway hyper-responsiveness, improve symptoms in term of severity and frequencies, and decrease β-agonists use and need for oral corticosteroids, which can reduce hospitalisations and urgent care visits (Klasco, 2005b).

1.1.38.5 Mechanism of action

Glucocorticoids inhibit the activity of a variety of inflammatory cell types. Also, oral inhaled corticosteroids suppress the late-phase allergic responses associated with chronic bronchial asthma (Brunton et al., 2006).

1.1.39 Formoterol

It is a long-acting β_2 agonist, which has an extended duration of action (up to 12 hrs) compared to short-acting β_2 agonists such as salbutamol, which are effective for 4–6 hrs. It is used in the treatment of both asthma and COPD. Formoterol is used as a symptom controller to supplement prophylactic corticosteroid therapy.

1.1.39.1 Physical properties

Formoterol is a white, almost white, or slightly yellow powder. It is slightly soluble in water and in isopropyl alcohol, practically insoluble in acetonitrile and soluble in methanol. A 0.1% solution in water has a pH of 5.5 to 6.5 (Sweetman et al., 2006).

It has the chemical name (±)-2´-Hydroxy-5´-[(RS)-1-hydroxy-2-fethyl] formanilide fumarate, and molecular formula $(C_{19}H_{24}N_2O_4)_2, C_4H_4O_4$ =804.9 Figure 1.36 (UK-DoH., 2008).

1.1.39.2 Pharmacokinetics

The initial response of formoterol is 1-3 minutes after inhalation and the peak response is 1-3 hours while after oral administration it is 20 minutes (SYMBICORT®–Product_Information, 2004).

It has a duration of 8-12 hrs for single inhaled dose and oral dose is 5-8 hours (Klasco, 2005c).

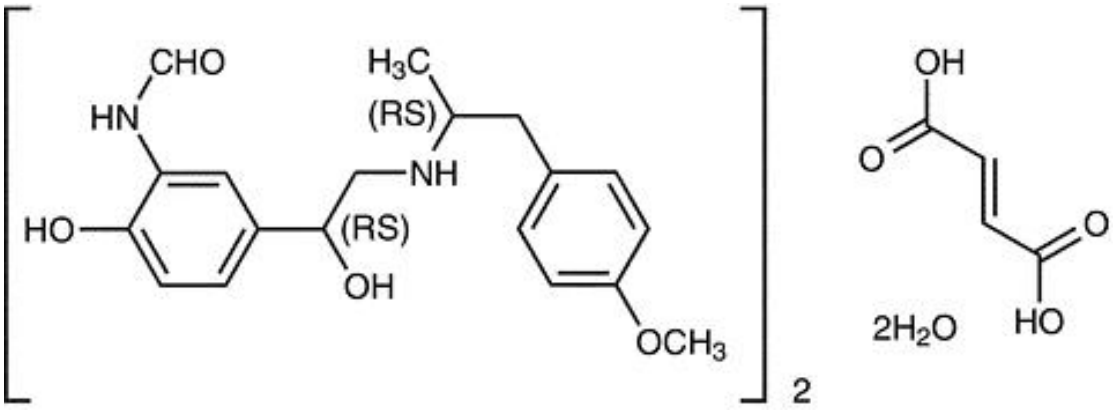

Figure 1.36 Formoterol structure

Formoterol is extensively metabolised in the liver (Brunton et al., 2006). The percentage of renal excretion is 60% and 16%-28% of a dose is excreted as unchanged drug (Klasco, 2005c). Its elimination half-life is 10 hours .

1.1.39.3 Monitoring parameters

The therapeutic efficacy can be monitored by pulmonary function test and reduction of mainly nocturnal symptoms. On the other hand, toxicity can be monitored using clinical signs such as heart rate, serum potassium level, blood pressure and blood glucose (Klasco, 2005c).

1.1.40 Beclomethasone Dipropionate

Beclomethasone is a synthetic, halogenated inhaled corticoid. Its oral inhaler is used in treatment of asthma and COPD (Brunton et al., 2006). Its chemical name is 9α-Chloro-11β,17α,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17,21-dipropionate, with molecular formula $C_{28}H_{37}ClO_{7,}$ and its molecular weight is 521.0 (Figure 1-37). It is a white or almost white, crystalline powder, practically insoluble in water, sparingly soluble in alcohol and freely soluble in acetone (Martindale et al., 2006).

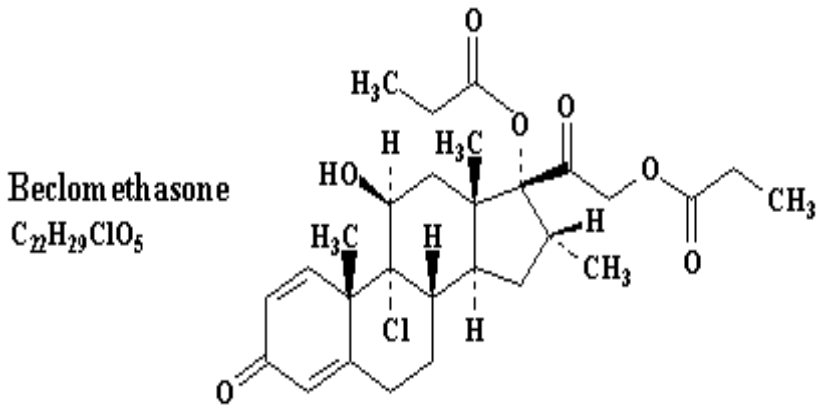

Figure 1.37 Chemical structure for beclomethasone.

1.1.40.1 Pharmacokinetics

The initial response of beclomethasone in asthmatic patients, if given by oral inhalation, is 1 to 4 weeks (Klasco, 2008). Its t_{max}, after administration of 4 puffs of hydrofluoroalkane-134a beclomethasone, (HFA-BDP), is 2103 pg/mL at 0.9 hours. Administration of the same dose of chlorofluorocarbon beclomethasone (CFC-BDP) is 1107 pg/mL at 1.4 hours (Lipworth, 1999). Administration of 4 puffs of HFA-BDP resulted in AUC of 8603 pg/mL, while administration of the same dose of CFC-BDP resulted in an AUC of 5755 pg/mL. This study concluded that the two formulations of BDP may not be equivalent on a microgram per microgram basis (Lipworth, 1999).

When it is given by oral inhalation, around 25% to 60% of each dose enters the bronchial airways. The lung absorption is rapid, with concentrations of 10 to 20 ng/g reached after 42 to 84 μg doses (Klasco, 2008). The total protein binding of beclomethasone is 87%. Metabolism sites are partially in the liver and extensively in the lung. It is rapidly hydrolysed by the lung. The main metabolites are beclomethasone-17-monopropionate, which is active, and meclomethasone alcohol, which is inactive. Its renal excretion is 10% to 15%,

and around 36% to 67% in faeces. It has an elimination half life 3 hours (Klasco, 2008).

1.1.40.2 Monitoring parameters

Monitoring parameters are similar to those for budesonide (see section 1.9.1.3)

1.1.40.3 Place In Therapy

Inhaled corticosteroids are considered as first-line therapy for managing persistent asthma and COPD in both children and adults (see section 1.9.1.4)

1.1.41 Beclomethasone vs Budesonide

Short-term studies have shown that budesonide is as effective as beclomethasone in the treatment of asthma. A number of double-blind trials have compared their efficacy in the management of asthma in adults receiving usual doses 200 to 800 μg/day (Rafferty et al., 1985, Keelan et al., 1984, Field et al., 1982, Willey et al., 1982), adults receiving high doses 800 to 1600 μg/day (Boe et al., 1989, Ebden et al., 1986, Svendsen et al., 1992) and children (Springer et al., 1987, Baran, 1987). These studies are however limited by short duration of therapy, between 2 and 8 weeks. But since there is a strong carry-over of anti-asthmatic effects with inhaled corticosteroids, it seems unlikely that such short-term trials could detect any treatment differences between the two medications. However, some studies demonstrated superiority of budesonide using a spacer device in the budesonide group and a conventional inhaler in the beclomethasone group (Field et al., 1982).

Reichel and co-workers (2001) compared an HFA-BDP breath-actuated inhaler (Qvar® Autohaler) with budesonide delivered by DPI, in patients with moderately severe, symptomatic asthma, incompletely controlled by on-going treatment with inhaled budesonide. The study demonstrated that HFA-BDP was clinically equivalent to twice the dose of budesonide.

AIMS AND OBJECTIVES

1.1.42 Background.

As has been shown in the literature review, drug delivery by the pulmonary route is a very important to give direct access of a drug to the targeted area. The inhalation route offers many advantages over systemic delivery. Currently, most drugs used in the treatment of asthma and COPD are delivered by inhalation.

However, there are still a number of questions and areas of concern. In these studies, some of these questions are investigated. As a first example Symbicort® Turbuhaler® is an inhaled drug, made up of a combination of budesonide and formoterol (SYMBICORT®_Product_Information, 2004) which shows synergistic effects in terms of reduction of asthma and COPD exacerbations (Pauwels et al., 1997a). Combining the anti-inflammatory corticosteroid budesonide and the rapid and long-lasting bronchodilator formoterol in the same device is designed to provide a simple, convenient and effective treatment. Budesonide is a glucocorticosteroid with high local anti-inflammatory effect (Brunton et al., 2006) and formoterol is a long acting and selective β_2- agonist (Brunton et al., 2006).

A major issue in the formulation is that budesonide is provided as a mixture of two epimers, 22R and 22S. The budesonide epimer R is known to be 2 to 3 times more potent than the budesonide epimer S (Ryrfeldt et al., 1982). As a result, it is essential to keep the epimeric ratio constant. In addition to this, the rate flow of each epimer may be different and therefore the pharmacological ratio may vary in delivery to the patient. Alongside this, it may be the case that formoterol and budesonide give differences in delivered drug ratio with change in flow rate.

The influence of flow rate on drug delivery by Turbuhaler® has been investigated in many studies (Jaegfeldt et al., 1987, Newman et al., 1991, Tarsin et al., 2004). However, these studies did not test the effect of flow rate on the two epimers and formoterol separately.

The third drug is beclomethasone, which is a synthetic halogenated inhaled corticoid. Its oral inhaler is used in treatment of asthma and COPD (Brunton et al., 2006).

On the other hand, beclomethasone, used in these experiments, is delivered by pMDIs, which are a convenient way of administering medication. But they emit an aerosol at high velocity and to be used properly they require coordination of inhalation and pMDI actuation. But even with an optimum technique, only < 15% of the emitted dose reaches the airways. As a result, spacers have been introduced. Spacer devices are intended to improve the efficacy of inhaled therapy by decreasing the need for coordination between actuation and inhalation by allowing deceleration and evaporation of propellant and by decreasing oropharyngeal deposition of therapy (Bisgaard et al., 2002). Many different spacers are currently available, some designed

to fit with one particular product, while others are intended for use with a variety of pMDIs.

In addition, CFCs have traditionally served as the propellant of choice for use with pMDIs. However, they deplete the ozone layer as noted by Molina and Rowland (1974). This has prompted a change to alternatives.

HFA134a has been approved by the Committee for Proprietary Medicinal Products (CPMP) under the European Medicines Evaluation Agency (EMEA), as an alternative to CFCs used in medicinal products (Cripps et al., 2000). As a result of the introduction of new propellants, many properties of the final products have changed. These changes may include extra-pulmonary deposition, taste and/ or lung deposition (Bisgaard et al., 2002).

1.1.43 Aim

In the case of oral inhalers, it is commonly recognised that particle size plays an important role in defining where the particles will deposit (Bisgaard et al., 2002). The main aim of the thesis is to examine the effect of the following factors: flow rate, spacers and drug formulation, on pulmonary delivery to the patient. In addition, a secondary aim is to examine the hypothesis of whether the result of a specific spacer with a given drug/ brand name can be extrapolated to other pMDIs or brand names for the same drug.

1.1.44 Objectives

- To determine the in-vitro performance of formoterol and the two epimers of budesonide under different flow rate conditions from a Turbuhaler® by determining the fine particle dose (FPD) and the mass

median aerodynamic diameter (MMAD) of Symbicort turbuhaler, using 28.3L/min and 60 L/min flow rate.

- To develop a sensitive and a simultaneous HPLC method for the analysis of formoterol and the two epimers of budesonide. Especially, it is required by the PhEuro (2007) that ratio of the two epimers (R/S) has to be within the range of 60-49/40-51%.
- To examine the effect of different type of spacers and drug formulations on the dose of beclomethasone delivered to the lungs and the throat deposition. Also, to measure the dose emitted from different brand names of beclomethasone formulations alone and attached to spacers using different parameters, including washed versus unwashed and the number of actuated doses.
- To compare the in-vitro aerosol deposition characteristics from different beclomethasone pMDIs with three common spacers using FPD and MMAD as parameters.

CHAPTER 2

MATERIALS AND METHODS

2 MATERIALS AND METHODS

APPARATUS AND INSTRUMENTATION

2.1.1 HPLC System

The HPLC SYSTEM was a 1050 Hewlett Packard series instrument which consisted of a HP 1050 UV-detector, autosampler pumping system (Hewlett Packard, Waldbronn, Germany).

2.1.2 Integrator

Prime integration software was used. (HPLC Technology, Ltd, Welwyn Garden City, UK)

2.1.3 Scanning UV detector

The UV spectrophotometer was a Hewlett Packard diode-array spectrophotometer 8452A (Hewlett Packard, Palo Alto, California, USA)

2.1.4 HPLC column

HPLC column was a C_{18} ODS2 Spherisorb 5 µm column 250mm X 4.6mm id. (Capital Analytical Ltd, Leeds, UK)

2.1.5 General laboratory apparatus

pH meter was Accumet® AB10, (Fisher Scientific, Pittsburgh, PA,USA).

The microbalance was Thermo CAH C-35 capable of weighing to 6 figures (Scientific and medical Products Ltd, Cheadle, UK)

The electronic balance was a Mettler AE240 (Mettler Toledo Ltd, Greifensee, Switzerland)

Ultrasonic bath (Telsonic Ltd, Basel, Switzerland).

Water purification system was PURELAB® Ultra (Vivendi Water Systems, Bucks, UK)

2.1.6 Apparatus used to determine dose emission and the particles size.

A GAST 1023 Pump, 0-100L/min (GAST, Brook Hampton, Doncaster, UK).

Electronic digital flow meter Model DFM (Copley Scientific Ltd. Nottingham UK).

Andersen MKII cascade impactor (Copley Scientific Ltd.).

Critical Flow Controller Model TPK. (Copley Scientific Ltd.).

Sampling Apparatus for DPIs.and MDIs (Copley Scientific Ltd.).

Next Generation Pharmaceutical Impactor (NGI) (Copley Scientific Ltd.).

Copley Inhaler Testing Data Analysis Software (CITDAS) (Copley Scientific Ltd).

MATERIALS

2.1.7 Organic modifiers for HPLC

Acetonitrile (Fisher Scientific, Loughborough, UK)

Methanol (Fisher Scientific)

Ethanol (Fisher Scientific)

2.1.8 Buffer salts

Potassium dihydrogen orthophosphate (BDH Laboratories, Poole,UK)

Sodium hydroxide (BDH Laboratories)

Orthophosphoric acid (BDH Laboratories)

2.1.9 Analytes and general chemicals used

Budesonide (Sigma, Gillingham UK).

Formoterol (Cipla Ltd, Kurkumbh, India).

Beclomethasone (Sigma).

Releasil Silicone spray (Dow Corning Ltd, Barry Glamorgan, UK.)

2.1.10 Filters

Glass microfibre filter grade GF/A 81mm (Whatman international Ltd, Kent, uk)

Glass microfibre filter grade GF/A 47mm (Whatman International Ltd)

Galss fiber filter type A/E 25 mm (Pall Corporation, Michigan, USA)

2.1.11 Pharmaceutical preparations.

A Symbicort 400/12 Turbohaler® containing budesonide 400 µg and formoterol fumarate 12 µg /metered inhalation, were obtained from (AstraZeneca UK Ltd Luton, UK)

A Clenil 250 Modulite® containing beclomethasone 250 µg /metered inhalation was obtained from (Trinity-Chiesi Pharmaceuticals Ltd, Highfield, UK).

A Clenil 100 Modulite® containing beclomethasone 100 µg /metered inhalation was obtained from (Trinity-Chiesi Pharmaceuticals Ltd,).

A Qvar100 Easi-Breathe® containing beclomethasone 100 µg /metered inhalation was obtained from (IVAX, Harlow, UK)

Qvar® 100 aerosol inhalation, containing beclomethasone 100 µg /metered inhalation was obtained from (IVAX)

Beclazone 250 Easi-Breathe® containing beclomethasone 250 µg /metered inhalation was obtained from (IVAX,)

Beclazone 100 Easi-Breathe® containing beclomethasone 100 µg /metered inhalation was obtained from (IVAX)

Becloforte aerosol inhalation, containing beclomethasone 250 µg /metered inhalation was obtained from (GlaxoSmithKline Uxbridge, UK)

Becotide 100 aerosol inhalation, containing beclomethasone 250 µg /metered inhalation was obtained from (GlaxoSmithKline)

2.1.12 Spacer devices

AeroChamber® Plus AeroChamber® Max were obtained from (GlaxoSmithKline)

Optimizer® was obtained from (IVAX)

ANALYTICAL METHOD VALIDATION

The aim of validation of analytical methods is to display that the methods are appropriate for their intended function. Furthermore, the validation of the analytical methods demonstrates the reliability and performance in different conditions. Method validation for pharmaceuticals in the developed countries is now controlled by guidelines set out by the International Conference on Harmonization (ICH) guideline 2005 (HT Guideline, 2005). The explanations of these measurements are indicated below.

2.1.13 Specificity

Specificity is the capacity of an analytical procedure to assess unequivocally the analyte in the presence of other components, which may include impurities, degradation or any substance which may interfere with the analyte.

2.1.14 Linearity

In analytical science, the most easily used relationship is where a change is measured with respect to a primary variable, e.g. concentration, and the result is a predictable linear response. A linear relationship should be examined throughout the range of the analytical method. At the initial stage, a relation should be elicited from the test results from standards that are directly obtained, or by other means obtained from a well defined mathematical equation, thus reflecting the relationship between the concentration of an analyte in samples and signal height or peak area as a function of analyte concentration. According to the ICH guidelines, the linearity should be determined by at least a series of five different concentrations with at least replicate measurement (HT Guideline, 2005).

It is very common for the linearity to be evaluated graphically with or without mathematical evaluation. The evaluation is often made by visual inspection of a plot of the response, such as a signal height or peak area as a function of analyte concentration. If a relation is required, more mathematical tests can be preformed and a linear relationship established, such as correlation coefficient, y-intercept and slope of the line.

If the equation of the line is ideal, it should have an intercept close to zero. If the intercept is far from zero, then the effect of a non-zero intercept should be reconsidered in the method.

2.1.15 Accuracy

The accuracy of an analytical procedure expresses the degree of agreement between the test result generated and the true value.

2.1.16 Precision

The precision is defined by the ICH Harmonised Tripartite Guideline (HT Guideline, 2005) as “the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions”. The precision should be tested under the same working conditions. The relative standard deviation (RSD) is usually used to express the precision, although variance or coefficient of variation has been used in the past.

Many terms are used to express precision, such as intermediate precision and reproducibility.

2.1.16.1 Intermediate precision

Intermediate precision is a secondary term used to describe precision of the analytical procedure which is repeated on different days. It is also used to indicate the robustness of the assay over time.

2.1.16.2 Reproducibility

Reproducibility expresses the precision in terms of the closeness between different laboratories or different columns or instruments on the same or different sites.

2.1.17 Limit of detection

It is the lowest amount of analyte which can be detected within a certain criteria. One of the simple ways to obtained this is by measuring the response against the noise and 3 to 1 signal to noise. An alternative is to use the standard deviation to determine the limit of detection (LOD) (Guideline, 1996).

$$LOD = \frac{3.3\sigma}{S} \quad \text{.....Equation 2-1}$$

where σ = standard deviation of Y-intercept

S= slope of calibration curve.

σ was calculated using the following equation:

$$\sigma = \frac{\sqrt{\sum(\hat{y}i - yi)^2}}{n - 2} \quad \text{..... Equation 2-2}$$

where $\hat{y}i$ is the average, yi is (number1,number2,…) and n is the sample size

2.1.18 Limit of quantitation

The limit of quantitation (LOQ) is the lowest concentration of analyte in the injected sample that can be detected and precisely quantified. There are many methods to express the LOQ. The simplest is to find the concentration which give 10 time of the signal to the noise. The other method is represented by the following equation:

$$LOQ = \frac{10\sigma}{S} \quad \text{.....Equation 2-3}$$

Where σ = standard deviation of Y-intercept

S= slope of calibration curve.

2.1.19 Robustness

Robustness examines the reliability of the analytical procedure when small changes in the method parameters are carried out, such as pH, variation in mobile phase composition, column temperature and flow rate.

METHODS

2.1.20 Preparation of buffer

The suitable amount of buffer salt was accurately weighted, then transferred to a volumetric flask and Mili-Q water was added to made up to the desired volume.

2.1.21 Preparation of HPLC mobile phase

In order to avoid the volume contraction of the mobile phase which is associated with addition of aqueous solution to organic solution, the two phases were accurately measured separately then mixed together. The pH of buffer was adjusted to the apparent pH, using the appropriate acid. The mobile phase was filtered through a 0.45 µm filter under vacuum to remove any unwanted particles which may block the HPLC system. The mobile phase was degassed using an ultrasonic bath under vacuum for 10 min before use. The pH meter was calibrated regularly using a commercial product to avoid inaccurate pH reading.

2.1.22 Preparation of washing solution for inhalation sampling apparatus

In order to extract the drug from the apparatus, the washing solution was chosen to be compatible with the HPLC condition. The washing solution was prepared by mixing acetonitrile and water in the ratio of 70:30 v/v. The two phases were measured separately, then mixed together.

BUDESONIDE AND FORMOTEROL ANALYTICAL METHOD DEVELOPMENT AND VALIDATION

2.1.23 Objective of HPLC method development

The main objective of this study was to develop a sensitive and simultaneous HPLC method for the analysis of formoterol and the two epimers of budesonide. The budesonide epimer R is known to be 2 to 3 times more potent than the budesonide epimer S (Ryrfeldt et al., 1982). Furthermore, as required by the PhEuro, the ratio of the two epimers (R/S) has to be within the range of 60-49/40-51 (PhEuro, 2007). Therefore the analyst is required to have methods for these assays resulting in 2 methods being used. It would be a considerable advantage if an HPLC method could be found to replace the two separate methods from both a saving viewpoint and this an objective of a part of the research programme.

2.1.24 Method development for budesonide and formoterol

A literature review was carried out prior to method development. This is summarised for formoterol in Table 2.1 and budesonide in Table 2.2. As the tables show, there is only one method which has separated formoterol and budesonide in one single method, developed by Assi et al (2006). However, it does not separate the two budesonide epimers. Consequently, there is a need for a single method to separate formoterol and its two epimers, as this is required by the PhEuro to give a fixed ratio between the two epimers.

2.1.25 Determination of optimal detection wavelengths

Before carrying out the HPLC separation, the UV spectrometer was used to determine the optimal wavelength for formoterol and budesonide.

Table 2.1 The chromatographic conditions and parameters of the three HPLC formoterol assay methods

Parameters	Method (Akapo et al., 2003)	Method[b] for formoterol and budesonide (Assi et al., 2006)
Column	Alltech Alltima C_{18} 5 mm silica column (15 cm x 4.6 mm)	250mm×4.6mm i.d. (5 µm particle size) Spherisorb C_{18} column (Waters, UK)
Mobile phase	ammonium acetate (50 mM; pH 5.0) and methanol in the ratio 65:35 v/v	acetonitrile–5mM sodium dihydrogen orthophosphate, pH 3 (60:40%, v/v).
Flow rate (ml/min)	1.0	1.5
Wavelength (nm)	242	214

Table 2.2 The chromatographic conditions and parameters of the three HPLC budesonide assay methods

Parameters	EtOH method[a]	European Pharmacopoeia method[b]	Et+ACN+ Buffer method[c]
Column	Hypersil C_{18} column 5 mm 30 cm4.6 mm i.d.	Hypersil C_{18} column, 5mm 12 cm 4.6 mm i.d.	Hypersil C_{18} 15 cm4.6 mm i.d. column, 5 mm
Mobile phase	Ethanol–water (43:57, v:v)	Acetonitrile–phosphate buffer (pH 3.2; 25.6 mM) (30:70, v:v:v)	Ethanol–acetonitrile –phosphate buffer (pH 3.4; 25.6 mM) (2:30:68, v:v:v)
Flow rate (ml:min)	1	1.5	1.5
Wavelength (nm)	254	240	240
Retention time (min)	R-epimer 16 min S-epimer 18 min	R-epimer 16 min S-epimer 18 min	R-epimer 18 min S-epimer 20 min
Limit of detection	not mentioned	not mentioned	(0.3 µg/ml) >1 µg/ml

a. (Roth et al., 1980)

b-(Pharmacopoeia, 1997)

C-(Hou et al., 2001)

2.1.26 HPLC method optimisation

In the optimisation of the HPLC method for the detection of budesonide and formoterol, a trial and error strategy was used. Variable pH, organic modifier and buffer capacities were examined in order to reach the optimal retention time and the best separation for the two epimers.

2.1.27 Result

2.1.27.1 Determination of the optimal detection wavelengths

By using the UV-spectrometers, two wavelengths were selected (Figures 2.1a and 2.1b). The best wavelengths were 214 nm for formoterol and 242 nm for budesonide. From the UV scan, it was clear that to obtain maximum sensitivity from assay and to use only one assay procedure, ,the optimum method would be to have one HPLC procedure but to use two detection wavelengths. In many cases, the change of wavelength within method is not possible, because of large shift in the base line between the changes. However, the wavelength change in this case resulted in a minor shift which did not affect the detection and quantitation assay. In addition, the assay procedure provided very good resolution between the components and therefore there was no interference at the wavelength changeover, as shown in Figure 2.5.

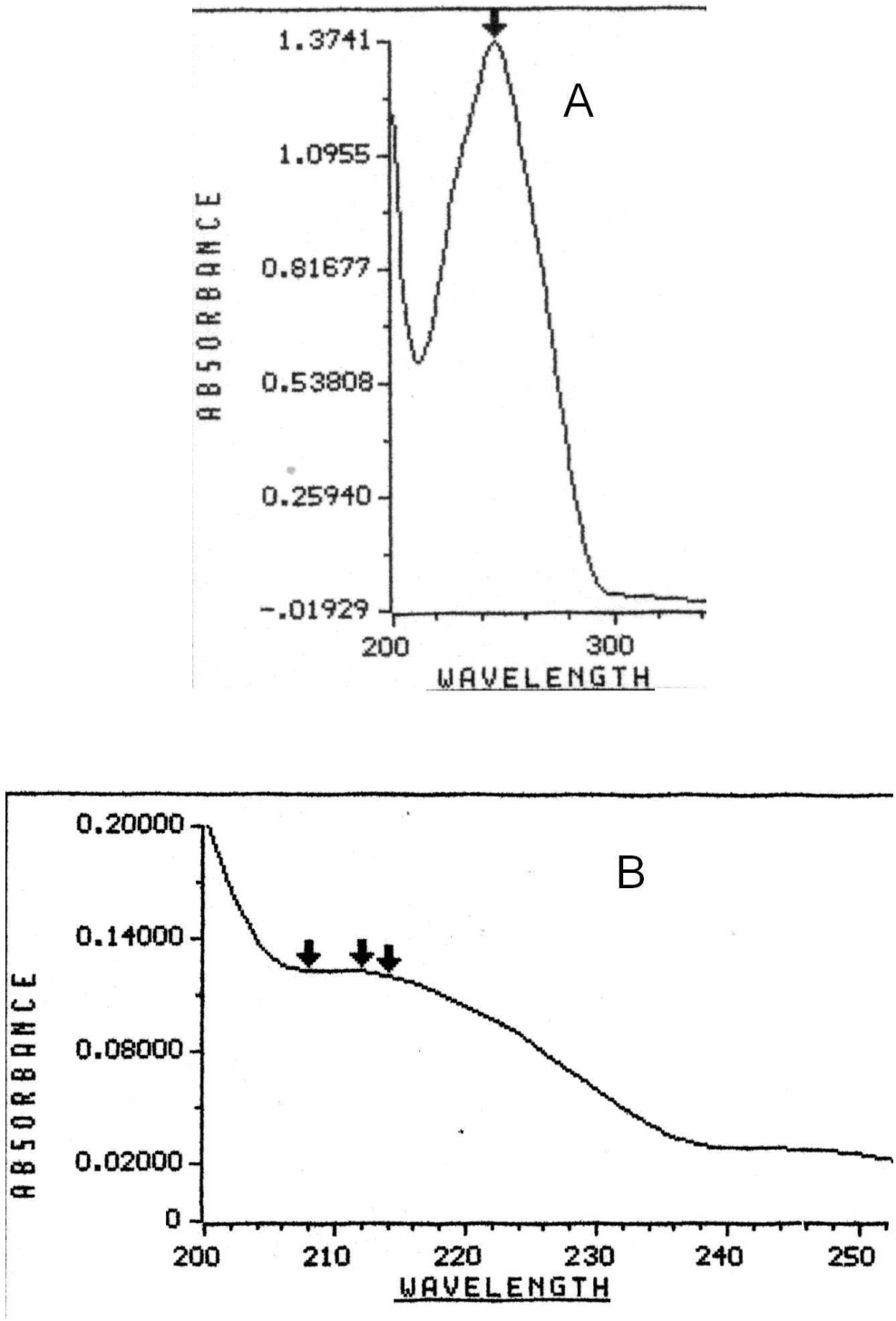

Figure 2.1 A- UV-spectrometers for budesonide 40µg/ml (Acetonitrile: water 70:30 v/v) B- UV-spectrometers for formoterol 1.17 µg/ml (Acetonitrile: water 70:30 v/v)

2.1.27.2 HPLC methods development.

As mentioned, a method has been used previously to detect budesonide and formoterol simultaneously. But it detects the budesonide and formoterol without separation of the two epimers (Assi et al., 2006). PhEuro (2007) requirs keeping a fixed ratio between the two epimers.

In order to find a suitable method for the separation of the drug and epimers different organic modifier concentrations were tested, as shown in Figure 2.2. The two factors which had the largest effect on resolution were organic modifier concentration and buffer capacity. The best concentration was found to be 40:60 v/v of Acetonitrile: buffer and the best buffer capacity was 7.5 mM, as shown in Figure 2.3, which allowed separation.

Table 2.3 The chromatographic conditions for budesonide and formoterol

Parameters	HPLC budesonide and formoterol
Column	reversed-phase 5 µm hypersil C18 column, 250 X 5 mm
Mobile phase	acetonitrile, and buffer 7.5 mM Potassium dihydrogen phosphate (40:60 v/v) pH 3
Flow rate (ml:min)	1 mL/min, isocratically.
Wavelength (nm)	214/ 240
Temperature	Room temperature
Injection volume	100 µL

The two epimers were very sensitive to any change in the organic modifier but formoterol was not. On the other hand, budesonide epimers were less sensitive to buffer capacity change. Table 2.3 shows the final HPLC conditions.

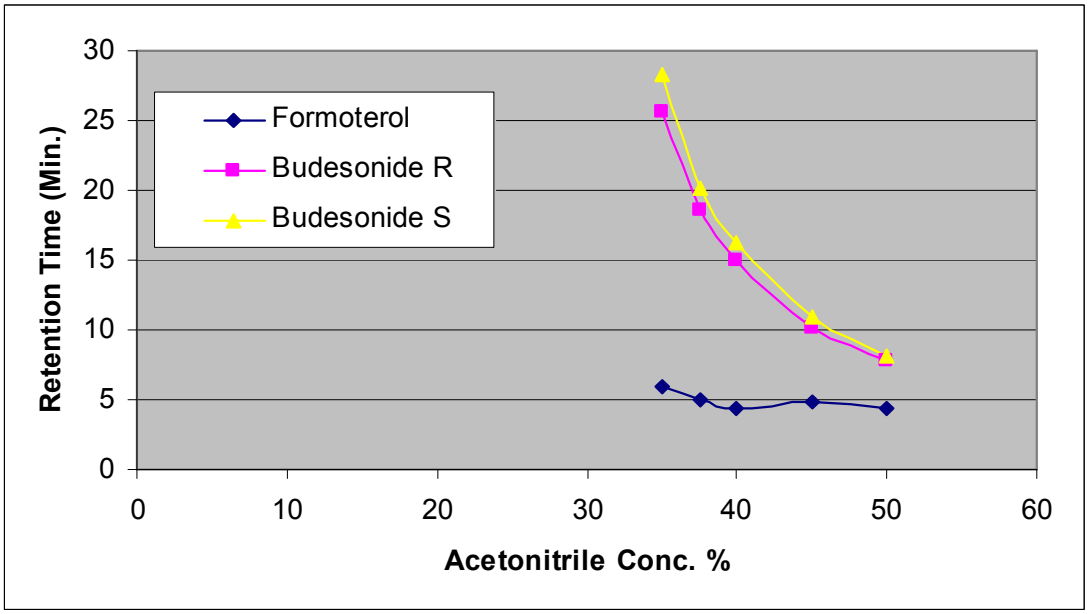

Figure 2.2 Effect of organic modifier on retention time

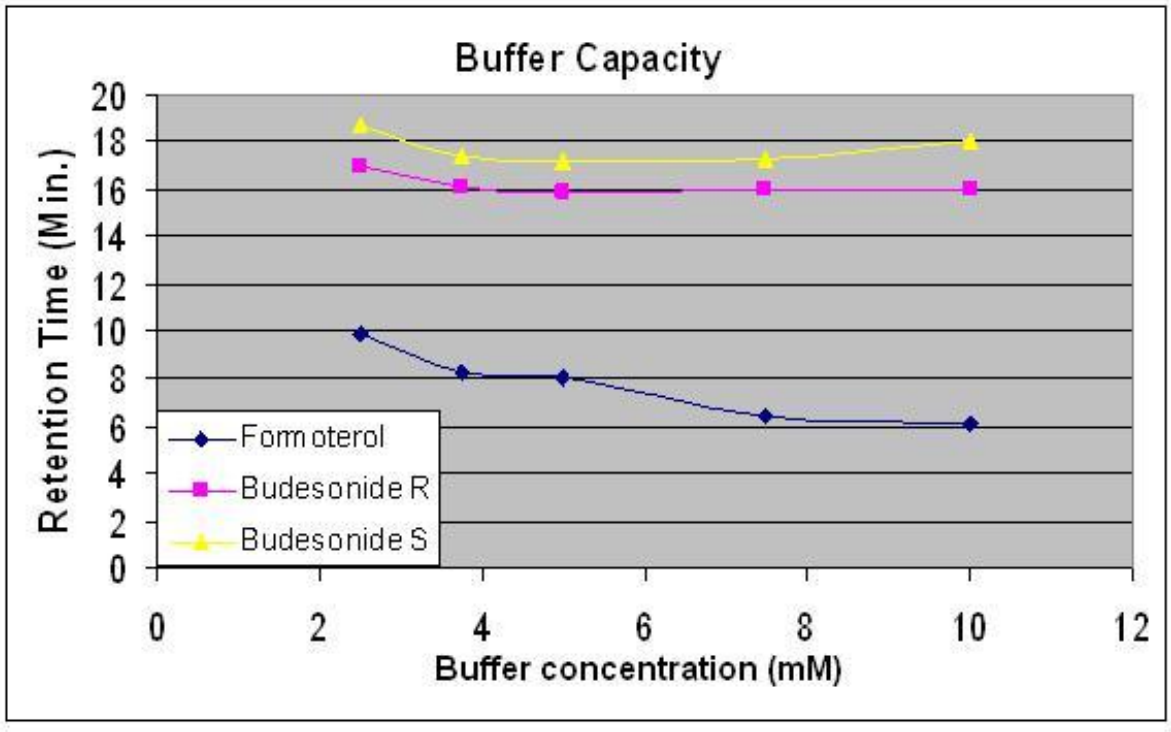

Figure 2.3 Effect of buffer capacity on retention time

2.1.27.3 Analytical method validation.

In this study, the HT Guideline 2005 (HT Guideline, 2005) was followed to validate the method.

2.1.27.3.1 Selectivity

The method developed was shown to be selective for formoterol and both budesonide epimers, as shown in Figure 2.4. Figure 2.5 confirmed that there were no interfering peaks due to the blank Acetonitrile: water (70:30 v/v).

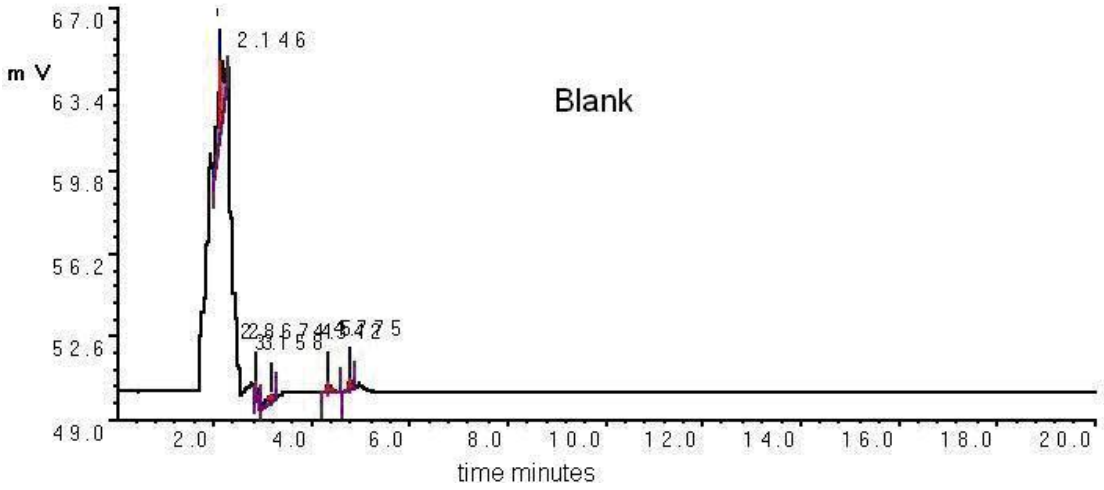

Figure 2.4 Chromatographic profiles of blank (Acetonitrile: water 70:30 v/v). For full HPLC conditions (see Table 2.3).

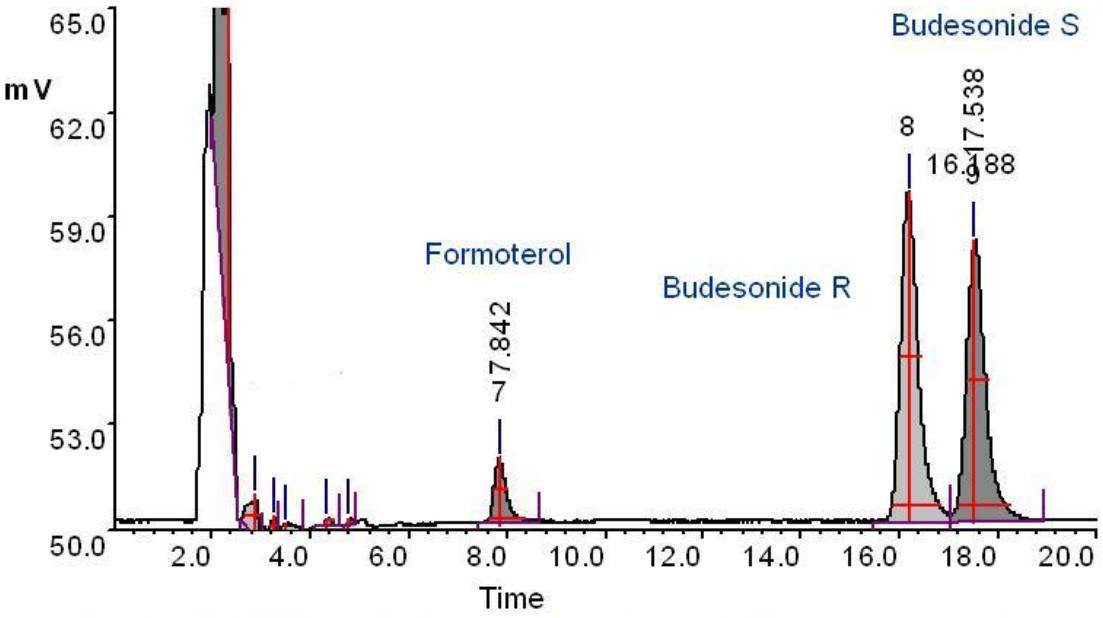

Figure 2.5 Chromatographic profiles of formoterol and the two epimers of budesonide. For full HPLC conditions (see Table 2.3)

2.1.27.3.2 Linearity

Peak heights and peak areas were linear over the range of 20.46 – 272.5 µg/ml and 0.88 – 11.72 µg/ml for both budesonide and formoterol, respectively. The calibration solutions were diluted with a solution of acetonitrile: water 70:30 v/v. The linear response for formoterol yielded a regression equation of (y = 179.3 x - 28.88) with a correlation coefficient (R^2 = 0.998 n=10) and (y = 8.879 x + 1.041) (R2 = 0.9998 n=10) for peak area and peak height, respectively. A linear response for the two epimers was also given and budesonide R gave a regression equation of (y = 49.86 x - 110.9) with a correlation coefficient (R^2 = 0.999 n=10) for peak area and (y = 1.684 x + 13.86) (R2 = 0.9965 n=10) for peak height. Secondly, budesonide S yielded a regression equation (y =42.84 x -125.93) (R2=0.9983 n=10) for peak area and (y =1.28X +12.43) (R^2=0.99 n=10) for peak height (Figure 2.6).

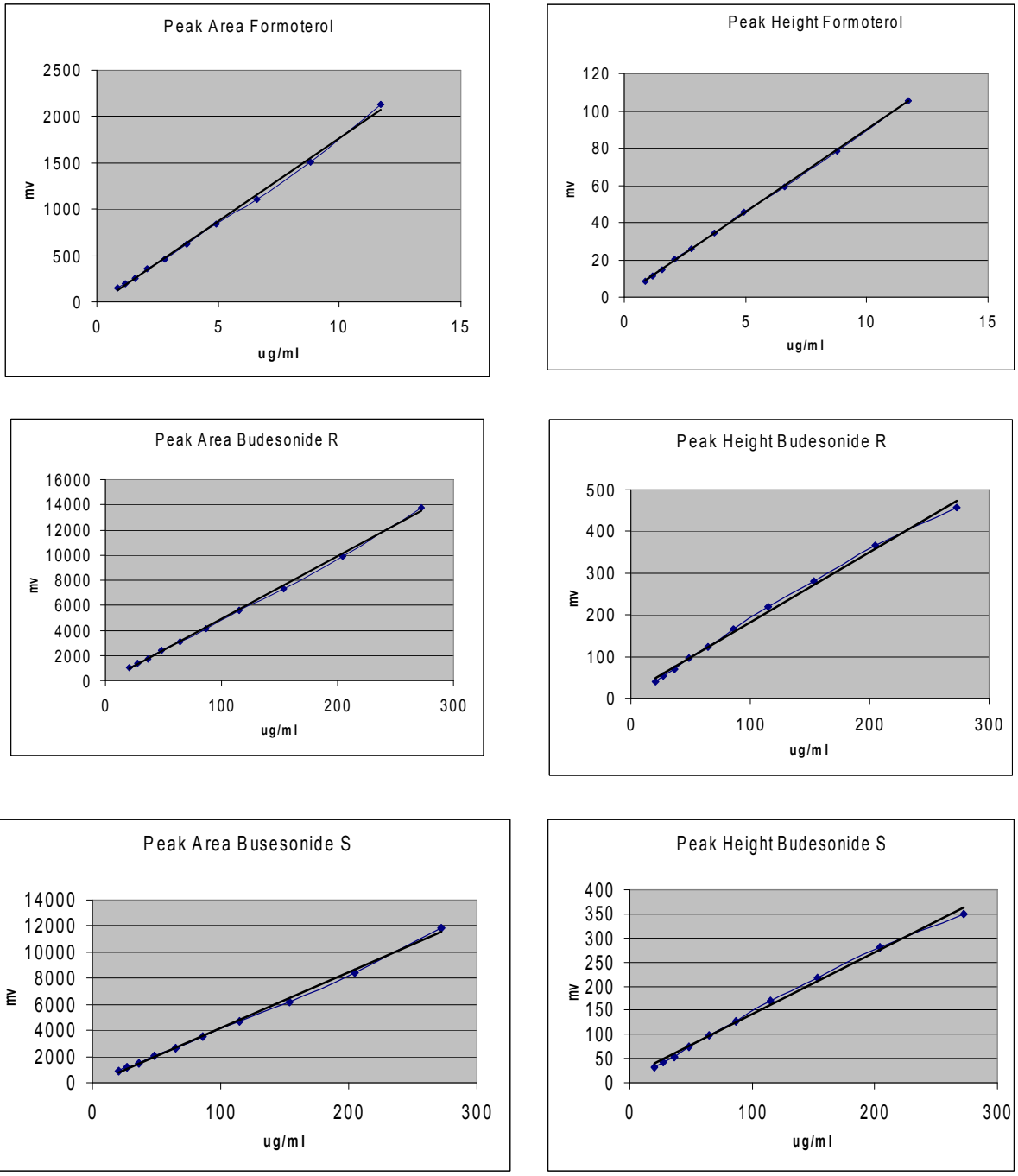

Figure 2.6 Linearity plot for budesonide epimers and formoterol. For full HPLC conditions (see Table 2.3).

2.1.27.3.3 Accuracy

The data in Table 2.4 show very good recoveries at all levels. The average recoveries for three concentrations, low, medium and high in the linear range 0.9-11.5 ug/ml, 50–250 ug/ml and 50–250 ug/ml for formoterol, budesonide R, and budesonide S, respectively, were used to examine the accuracy (*n* = 5 for each level). The resultant statistic shows that the method has good accuracy. The method was therefore suitably accurate for use in the assay of formoterol, budesonide R and budesonide S

Table 2.4 Accuracy data for budesonide R, budesonide S and formoterol content (*n*= *5*)

Budesonide R		
Concentrations levels (ug/ml)	Accuracy (%)	
	Mean	RSD
High 250	98.2	0.04
Medium 125	97.9	0.08
Low 50	98.1	0.2
Budesonide S		
Concentrations levels (ug/ml)	Accuracy (%)	
	Mean	RSD
High 250	98.7	0.1
Medium 125	98.5	0.08
Low 50	97.9	0.02
Formoterol		
Concentrations levels (ug/ml)	Accuracy (%)	
	Mean	RSD
High 11.5	98.2	0.08
Medium 6	97.8	0.09
Low 0.9	97.5	0.2

RSD: Relative standard deviation

2.1.27.3.4 Precision

The RSD of peak area responses for five standard injections was 0.257%, 0.084%, 0.212% for formoterol, budesonide R and budesonide S, respectively and these results meet the ICH Guideline. Furthermore, the retention time and peak height were tested and the results are shown in Table 2.5.

Table 2.5 Precision of assay method. For full HPLC conditions (see Table 2.3).

Run	Substance	Retention time (min)	Area (mv)	Height (mv)
First run	Formoterol	7.808	150.4	8.600
	Budesonide R	15.22	1,002	40.86
	Budesonide S	16.38	849.2	32.01
Second run	Formoterol	7.804	150.5	8.626
	Budesonide R	15.213	1,003	40.89
	Budesonide S	16.37	852.2	32.05
Third run	Formoterol	7.804	151.3	8.665
	Budesonide R	15.21	1,003.	40.93
	Budesonide S	16.37	849.0	32.06
Forth run	Formoterol	7.796	151.09	8.657
	Budesonide R	15.21	1,003.	40.95
	Budesonide S	16.37	851.9	32.09
Fifth run	Formoterol	7.804	150.7	8.662
	Budesonide R	15.22	1,004	40.96
	Budesonide S	16.38	852.9	32.09
Formoterol	AVG	7.803	150.8	8.642
	STDEV	0.004	0.387	0.028
	RSD	0.056	0.257	0.326
Budesonide R	AVG	15.22	1,003	40.92
	STDEV	0.002	0.847	0.042
	RSD	0.014	0.084	0.103
Budesonide S	AVG	16.37	851.0	32.06
	STDEV	0.004	1.806	0.033
	RSD	0.027	0.212	0.103

2.1.27.3.5 Intra-day precision.

The Intra-day precision was tested. A series of five runs have been done at five different times on the same day. The results are summarised in the Table 2.6.

Table 2.6 Repeatability of assay method. For full HPLC conditions (see Table 2.3).

Run	Substance	Retention Time (min)	Peak Area (mv)	Peak Height (mv)
First run	FORMOTEROL	7.830	621.1	34.24
	BUDESONIDE R	15.23	4133	164.0
	BUDESONIDE S	16.41	3508	128.0
Second run	FORMOTEROL	7.830	622.8	34.10
	BUDESONIDE R	15.23	4134	164.4
	BUDESONIDE S	16.41	3509	128.3
Third run	FORMOTEROL	7.830	623.1	34.14
	BUDESONIDE R	15.23	4139	164.5
	BUDESONIDE S	16.41	3511	128.4
Forth run	FORMOTEROL	7.830	621.9	34.12
	BUDESONIDE R	15.24	4141	164.7
	BUDESONIDE S	16.42	3512	128.5
Fifth run	FORMOTEROL	7.830	622.8	34.20
	BUDESONIDE R	15.23	4141	164.9
	BUDESONIDE S	16.41	3514	128.7
FORMOTEROL	Average	7.830	622.4	34.16
	SD	0.00	0.84	0.06
	RSD	0.02	0.13	0.17
BUDESONIDE R	AVERAGE	15.23	4138	164.5
	SD	0.00	3.57	0.32
	RSD	0.02	0.09	0.19
BUDESONIDE S	AVERAGE	16.41	3511	128.4
	SD	0.00	2.54	0.26
	RSD	0.02	0.07	0.20

SD: Standard DEVIATION, RSD: Relative standard deviation

2.1.27.3.6 Intermediate precision

The RSD value for intermediate precision performed on different days is summarised in Table 2.7.

Table 2.7 Intermediate precision of assay method

Run	Substance	Retention time (min)	Area (mv)	Height (mv)
First run	FORMOTEROL	7.630	2126	104.9
	BUDESONIDE R	15.14	13739.	459.1
	BUDESONIDE S	16.29	11822.	351.1
Second run	FORMOTEROL	7.620	2125	105.3
	BUDESONIDE R	15.13	13748	459.0
	BUDESONIDE S	16.28	11830	350.96
Third run	FORMOTEROL	7.610	2126	105.5
	BUDESONIDE R	15.12	13744	458.7
	BUDESONIDE S	16.27	11830	350.8
Forth run	FORMOTEROL	7.600	2128	106.2
	BUDESONIDE R	15.11	13740	455.0
	BUDESONIDE S	16.26	11846	347.6
Fifth run	FORMOTEROL	7.580	2130	106.2
	BUDESONIDE R	15.10	13755	457.3
	BUDESONIDE S	16.25	11853	349.4
FORMOTEROL	AVG	7.610	2127	105.6
	STDEV	0.02	1.70	0.59
	RSD	0.25	0.08	0.56
BUDESONIDE R	AVG	15.12	13745	457.8
	STDEV	0.01	6.69	1.73
	RSD	0.08	0.05	0.38
BUDESONIDE S	AVG	16.27	11836	350.0
	STDEV	0.01	12.62	1.50
	RSD	0.09	0.11	0.43

2.1.27.3.7 Robustness

From these results, the method was found to be robust and, as expected, the retention time decreased with increasing mobile phase flow rate. Finally, changes in pH of the buffer solution had little effect on the chromatographic profile of budesonide epimers. On the other hand, changes in pH of the buffer solution had a detrimental effect on formoterol retention time and peak area.

Table 2.8 Effect of flow rate on retention time

Flow rate ml/minute	Formoterol (min)	Budesonide R (min)	Budesonide S (min)
0.80	10.66	22.53	24.31
1	8.13	16.45	17.78
1.20	6.57	13.59	14.65

Table 2.9 Effect of pH on retention time and peak area

	Formoterol		Budesonide R		Budesonide S	
pH	Retention time (min)	Area	Retention time (min)	Area	Retention time (min)	Area
3	8.13	203.60	16.45	1702.58	17.78	1337.38
5	11.61	43.24	16.75	1464.31	18.05	1159.13

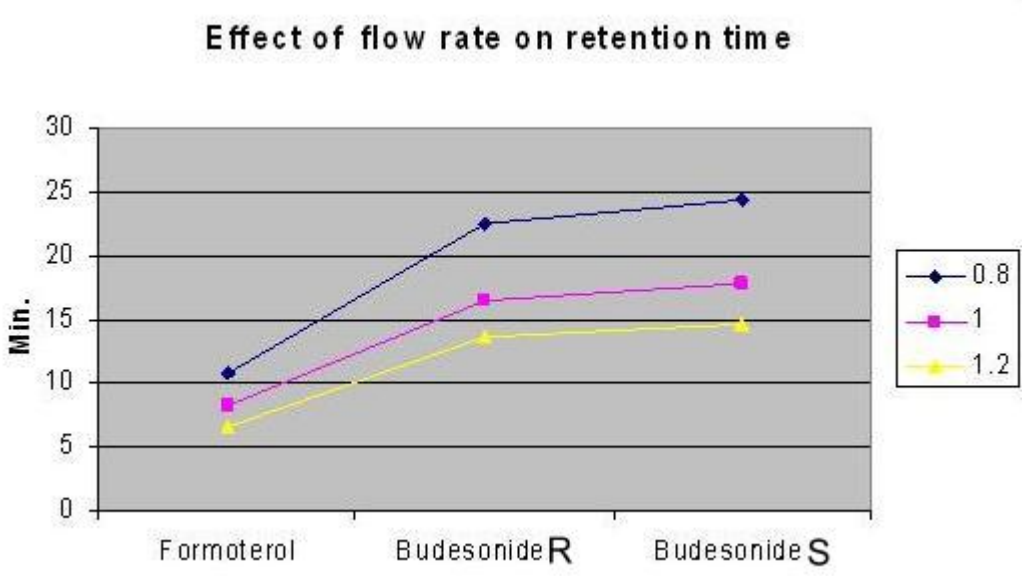

Figure 2.7 Effect of flow rate on retention time. For full HPLC conditions (see Table 2.3).

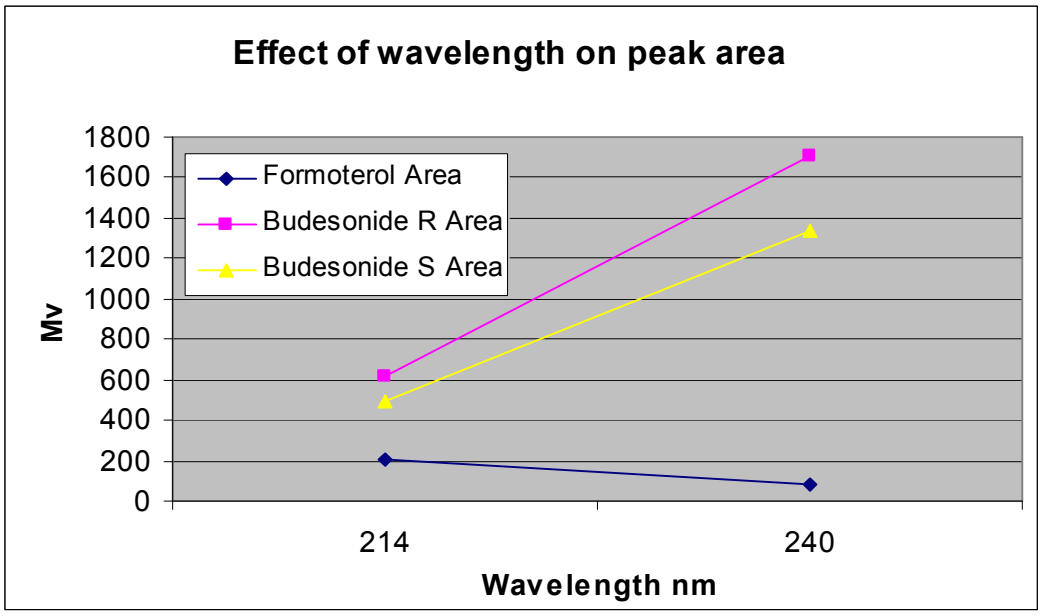

Figure 2.8 Effect of wavelength on peak area. For full HPLC conditions (see Table 2.3).

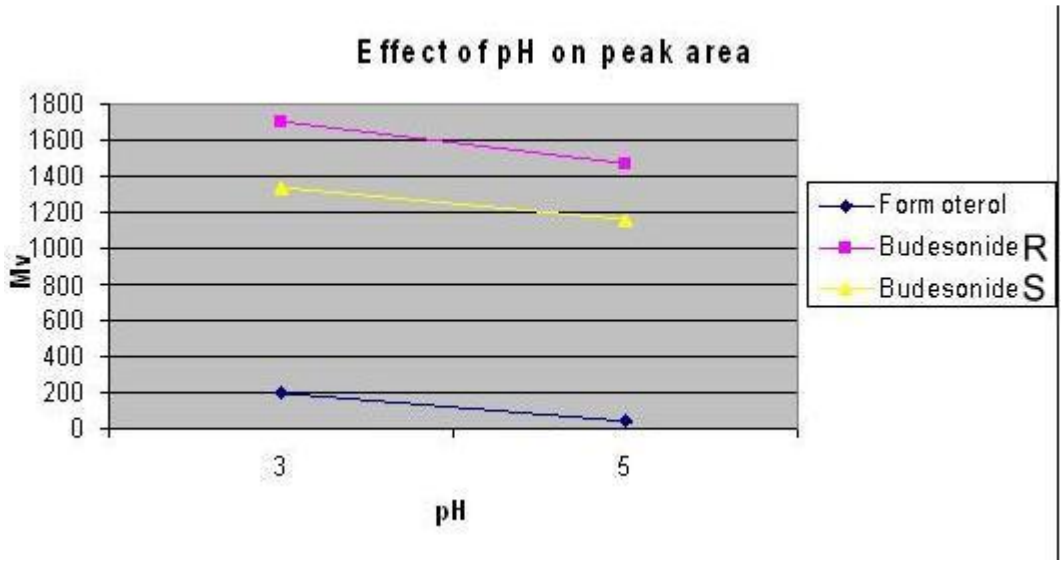

Figure 2.9 Effect of pH on peak area. For full HPLC conditions (see Table 2.3)

2.1.27.3.8 Limit of quantitation and limit of detection

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated from the average of the slopes and S.D. of the intercept of five calibration curves. The LOD and LOQ for the formoterol assay method were 5.401 ng/ml and 18 ng/ml, respectively. In addition, the LOD and LOQ for budesonide R assay method were 134.5 ng/ml and 448.4 ng/ml, respectively. Finally, the LOD and LOQ for the budesonide S assay method were 62.27 ng/ml and 207.57 ng/ml, respectively

2.1.28 Conclusion

An isocratic liquid chromatographic method has been described, which was optimised and validated for simultaneous qualitative and quantitative determination of formoterol fumarate and budesonide epimers. Acceptable assay precision and accuracy and excellent linearity were achieved In addition to its high sensitivity, and robustness, the proposed HPLC method

proved reliable in the determination of the budesonide and formoterol delivered from the Symbicort® Turbuhaler, as shown in Chapter 3. As a result, it has been shown that previously reported separated methods can be replaced by one only.

BECLOMETHASONE ANALYTICAL METHOD VALIDATION

An HPLC method was published by Elaraud (1997) has been adopted to analyse beclomethasone. Table 2.10 summarises the HPLC conditions. The HT Guidelines 2005 were followed to validate the analytical method (HT Guideline, 2005).

2.1.29 Result

2.1.29.1 Selectivity

The developed method demonstrates selectivity for beclomethasone. The blank (Acetonitrile: water 70:30 v/v) did not produce any peaks that interfered with the beclomethasone as shown in Figure 2.10.

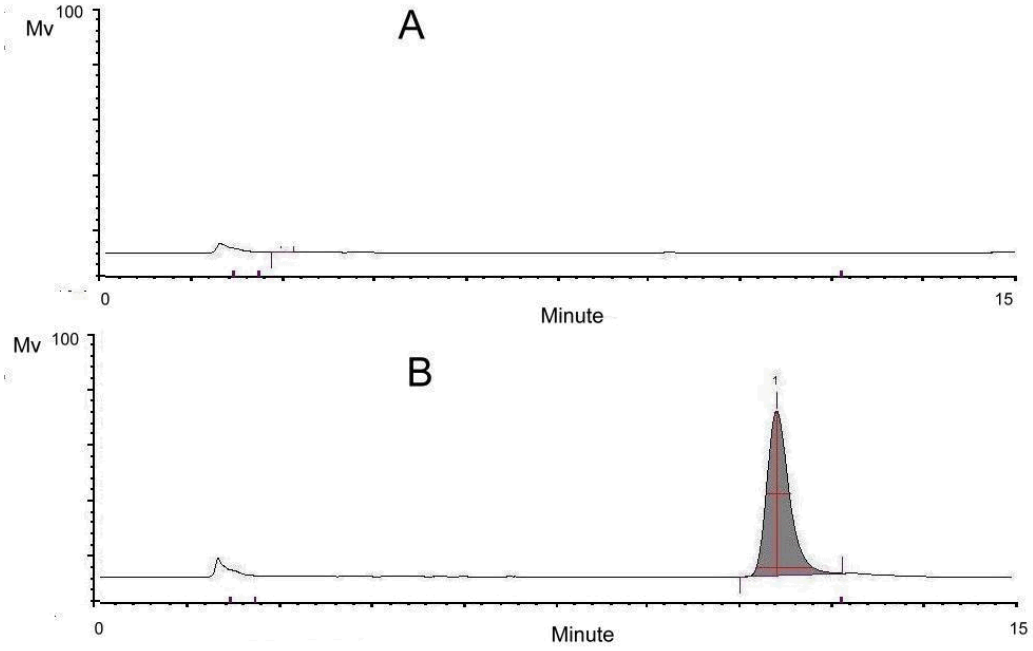

Figure 2.10 A- Chromatographic profiles of blank (Acetonitrile: water 70:30 v/v) B- Chromatographic profiles of beclomethasone. For full HPLC conditions see Table 2.10

Table 2.10 Chromatographic conditions for beclomethasone.

Parameters	HPLC beclomethasone
Column	reversed-phase 5 µm hypersil C_{18} 250 x 5 mm id
Mobile phase	50 mM Potassium dihydrogen phosphate pH 7 and acetonitrile (40:60 v/v)
Flow rate (ml/min)	1 mL/min, isocratically at room temp.
Wavelength (nm)	240
Temperature	Room temperature
Injection volume	100 µL
Limit of detection	9.20 ng/ml
limit of quantitation	28.0 ng/ml.

2.1.29.2 Linearity

Linear responses were obtained for beclomethasone over the concentration range 0.013 – 12.5 µg/ml, with a regression equation of (y = 66.93x + 0.6716) and a correlation coefficient R^2 = 0.9998 n=10 for peak Area Figure 2.10.

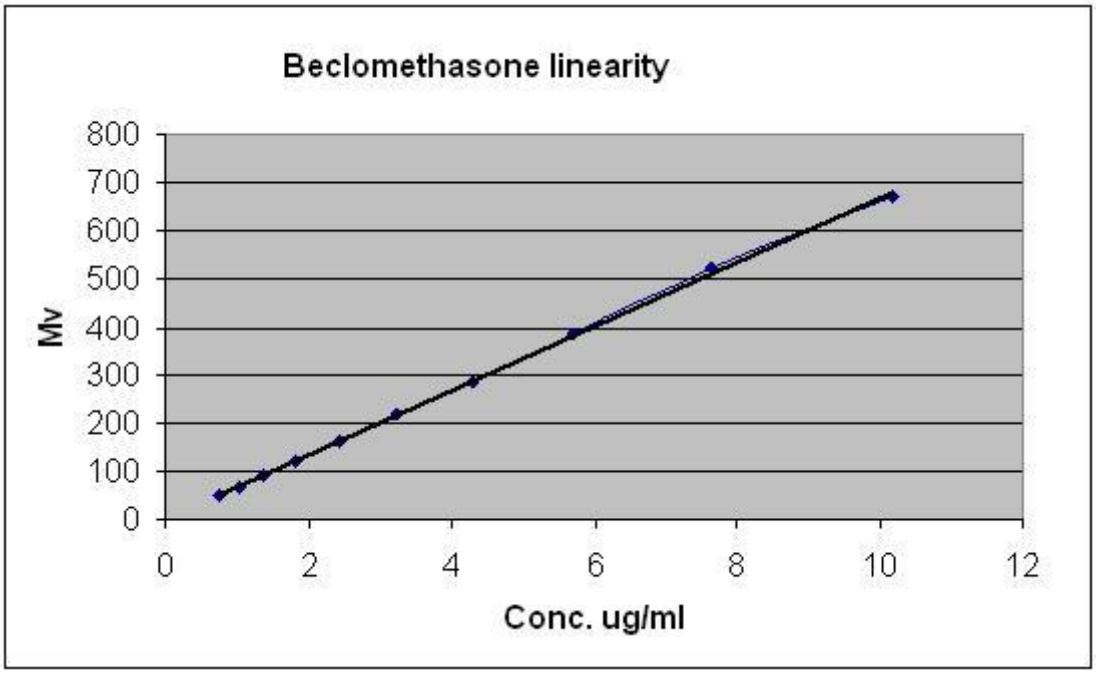

Figure 2.11 Linearity plot for beclomethasone. For full HPLC conditions (see Table 2.10).

2.1.29.3 Accuracy

The accuracy of the method was tested at three levels high 10 µg/ml, medium 6 µg/ml, and low 0.5 µg/ml (*n* = 5 for each level). The accuracy was estimated by the percent difference of the mean concentration determined from the known concentration. using the following equation

$$\text{Accuracy}=1-\frac{|\text{measured concentration - nominal concentration}|}{\text{nominal concentration}}X100$$ Equation 2-4

Values in Table 2.11 show that the method is suitable for determining the potency of beclomethasone.

Table 2.11 Accuracy data of Beclomethasone. For full HPLC conditions see Table 2.10

Beclomethasone		
Concentrations levels (ug/ml)	Accuracy (%)	
	Mean	RSD
High 10	99.1	0.02
Medium 6	98.7	0.06
Low 0.5	97.6	0.09

RSD: Relative Standard Deviation

2.1.29.4 Precision

The intra-day variation of the assay method was determined by replicate analysis of one concentration. The inter-day precision was evaluated by measuring replicates of the same samples over a period of 2 weeks (*n*=5). The precision was expressed as a percentage by calculating the intra- and inter-day RSD. All intra- and inter-day data are summarised in Table 2.12 and 2.13.

Table 2.12 Intra-day result of the Beclomethasone assay method

	Retention time	Peak Area
Run one	11.56	520.5
Run two	11.56	520.4
Run three	11.56	521.1
Run four	11.48	520.5
Run five	11.56	521.8
Average	11.55	520.9
Standard deviation	0.04	0.59
Relative standard deviation	0.32	0.11

Table 2.13 Result of inter-day of the Beclomethasone assay method

	Retention time	Peak Area
1st day	11.64	217.7
4th day	11.64	217.5
7th day	11.64	217.7
10th day	11.64	217.8
13th day	11.63	217.3
Average	11.64	217.6
Standard deviation	0.00	0.19
Relative standard deviation	0.04	0.09

2.1.29.5 Limit of quantitation and limit of detection

The LOD (S/N ≥ 3) was 9.2ng/ml and the (LOQ S/N ≥ 10) was 28ng/ml, where S is signal and N is noise.

CHAPTER 3

IN-VITRO AERODYNAMIC PARTICLE SIZE DISTRIBUTION OF THE SYMBICORT® TURBUHALER® AT DIFFERENT INHALATION FLOWS USING AN ANDERSON CASCADE IMPACTOR AND THE NEXT GENERATION CASCADE IMPACTOR.

3 IN-*VITRO* AERODYNAMIC PARTICLE SIZE DISTRIBUTION OF THE SYMBICORT® TURBUHALER® AT DIFFERENT INHALATION FLOWS USING AN ANDERSON CASCADE IMPACTOR AND THE NEXT GENERATION CASCADE IMPACTOR

OBJECTIVE

The objective of this section of the work was to determine the *in-vitro* performance of the formoterol and the two epimers of budesonide under different flow rate conditions. The fine particle dose (FPD) and the mass median aerodynamic diameter (MMAD) of 400/12 μg budesonide/formoterol) Symbicort® Turbuhaler® has been determined using 28.3L/min and 60 L/min flow rate.

INTRODUCTION.

Drug delivery via the pulmonary route is an important and rapidly rising area. Inhalation offers many advantages over the systemic delivery route. Currently, most drugs used in the treatment of asthma and airflow obstruction are delivered by inhalation. In the case of oral inhalers, it is commonly recognised that particle size plays an important role in defining where the particles will deposit in the lung (Bisgaard et al., 2002).

The regulators state that, all pharmaceutical dosage forms should guarantee that the drug is delivered in a safe and efficacious manner. Dose delivery is an important part of this because it is connected to both the safety and efficacy of the dosage form. For an inhaled dosage form, the amount of drug emitted from the device as well as the aerodynamic particle size, needs to be tested. In addition, it is essential that the in-*vitro* tests should be designed to mimic the patient's use as much as possible.

Table 3.1 Stage cut size (μm) for ACI for 28.3 and 60 L/min (UK., 2008) (USP, 2005)

Flow / Stage	28.3 L/min	60 L/min
Stage -1	Omitted	9.0 μm
Stage -0	Omitted	5.8 μm
Stage 0	9.0 μm	Omitted
Stage 1	5.8 μm	4.7 μm
Stage 2	4.7 μm	3.3 μm
Stage 3	3.3 μm	2.1 μm
Stage 4	2.1 μm	1.1 μm
Stage 5	1.1 μm	0.7 μm
Stage 6	0.7 μm	0.4 μm
Stage 7	0.4 μm	Omitted

As indicated previously one key analytical method which is used to assess inhalation, is cascade impaction, these impactors, including but not limited to the ACI and MSLIs, are widely used for particle size analysis and are recommended by both the USP and the Euro_Ph. (Mitchell et al., 2003). The ACI is manufactured by Copley Scientific in the UK and is an eight-stage cascade system intended for measuring the particle size distribution produced by pMDIs and DPIs. In order to test the effect of flow rate on particles size distribution, The ACI can be operated at different flow rates. However, it is essential to consider a modification in cut-points for each stage since the flow rate has an effect on the cut-points at each stage (see section 1.3.2.3.3.3). As an example the USP indicates that at 60 L/min, stages 0 and 7 are removed and replaced with two different stages, -0 and -1.

In the case of commercial inhaler, the Symbicort® Turbuhaler® shows flow–dependency for aerodynamic characteristics (Tarsin et al., 2004). Furthermore, many studies have demonstrated the effect of flow rate on the fine particles dose (Chrystyn, 2003, Al-fadhl, 2007).

Methods and instrumentation.

3.1.1 Equipment and Inhalation device

The device and the equipment used in this study are listed in Sections 2.1 and 2.2.

3.1.2 Instrumentation.

3.1.2.1 Procedure to set up Anderson Cascade Impactor for flow rate 28.3L/min.

The initial work was to prepare the ACI for measurement of the symbicort sample. All parts of the ACI and its stages including pre-separator were washed with acetone and dried. Furthermore, to ensure efficient capture of the particles and prevent bouncing the collection plates were sprayed with silicone (USP, 2005) which was allowed to air dry. The ACI stages were then assembled as described in the manufacture's manual which incorporates an after filter below the final stage to capture any fine particles that otherwise would escape from the apparatus Figure 3.1. A washing solution of 10 ml (acetonitrile: water 70:30, v/v) was placed into the preseparator to avoid re-entrainment of impacted particles larger than 10 µm which may interfere with other particle size groups. Figure 3.1 shows the positioning of the ACI, critical flow controller, and the vacuum pump. The mouthpiece adapter was attached to the end of induction port and the system was checked for airtightness. The

flow control valve was adjusted to achieve a steady flow through the system at the required rate 28.3L/min (±5%), which was measured by an electronic digital flow meter [Model DFM Copley Scientific Ltd]. According to the Pharmacopea method 4 L of air should be drawn through the inhaler for each determination and the sonic flow, the absolute pressure ratio P3/P2 < 0.5 was confirmed. The dry-powder inhaler was then prepared according to the patient leaflet instructions (see appendix A) and was discharged into the apparatus by opening the valve for the required time, 8.4 sec (±5%). The time was calculated according to the equation 4.1. Groups of five doses were selected randomly using random schedules, and each of these five doses was separately discharged into the apparatus from the device. Doses not used were discharged to waste using a flow rate of 90 L/min. The apparatus was then dismantled carefully to avoid any loss of material. The active ingredient was washed from the inner walls and the collection plate of each of the stages of the apparatus into an appropriate volume of the washing solution Table 3.2. The drug under test was extracted from the filter into the washing solution. In order to ensure complete extraction the filter was sonicated for three minutes in the washing solution. Also, the washing solution from the filter was further filtered through a 0.45 µm filter in order to remove any unwanted particles which may block the HPLC system.

3.1.2.2 Procedure to set up Anderson Cascade Impactor for flow rate 60L/min.

The procedure is identical to that above in section 3.3.2.1 except that, a manual procedure was used to replace stages 0 and 7 by -1 and -0. As a

result the cut-point would not be altered by using high flow rate. Furthermore, the valve closing time was decreased to 4 seconds using equation 4.1.

Table 3.2 Washing volume of 70% acetonitrile for ACI stages.

Flow / Stage	28.3 L/min	60 L/min
Induction port	20 ml	20 ml
pre-separator	10	10 ml
Stage -1	Omitted	5 ml
Stage -0	Omitted	10 ml
Stage 0	5 ml	Omitted
Stage 1	10 ml	10 ml
Stage 2	10 ml	10 ml
Stage 3	10 ml	10 ml
Stage 4	10 ml	10 ml
Stage 5	10 ml	5 ml
Stage 6	5 ml	5 ml
Stage 7	5 ml	Omitted
Filter	10 ml	10 ml

3.1.2.3 Procedure to set up Next Generation Cascade Impactor for flow rate 60L/min.

The apparatus was assembled with the pre-separator as shown in Figure 3.2. Then cups were placed into the apparatus. In addition all cups were sprayed with silicone in order to reduce the re-entrainment of impacted particle. Then the cups were placed in the bottom frame and the impactor lid was closed with the seal body attached, and the handle was operated to lock the impactor together so the system was airtight.

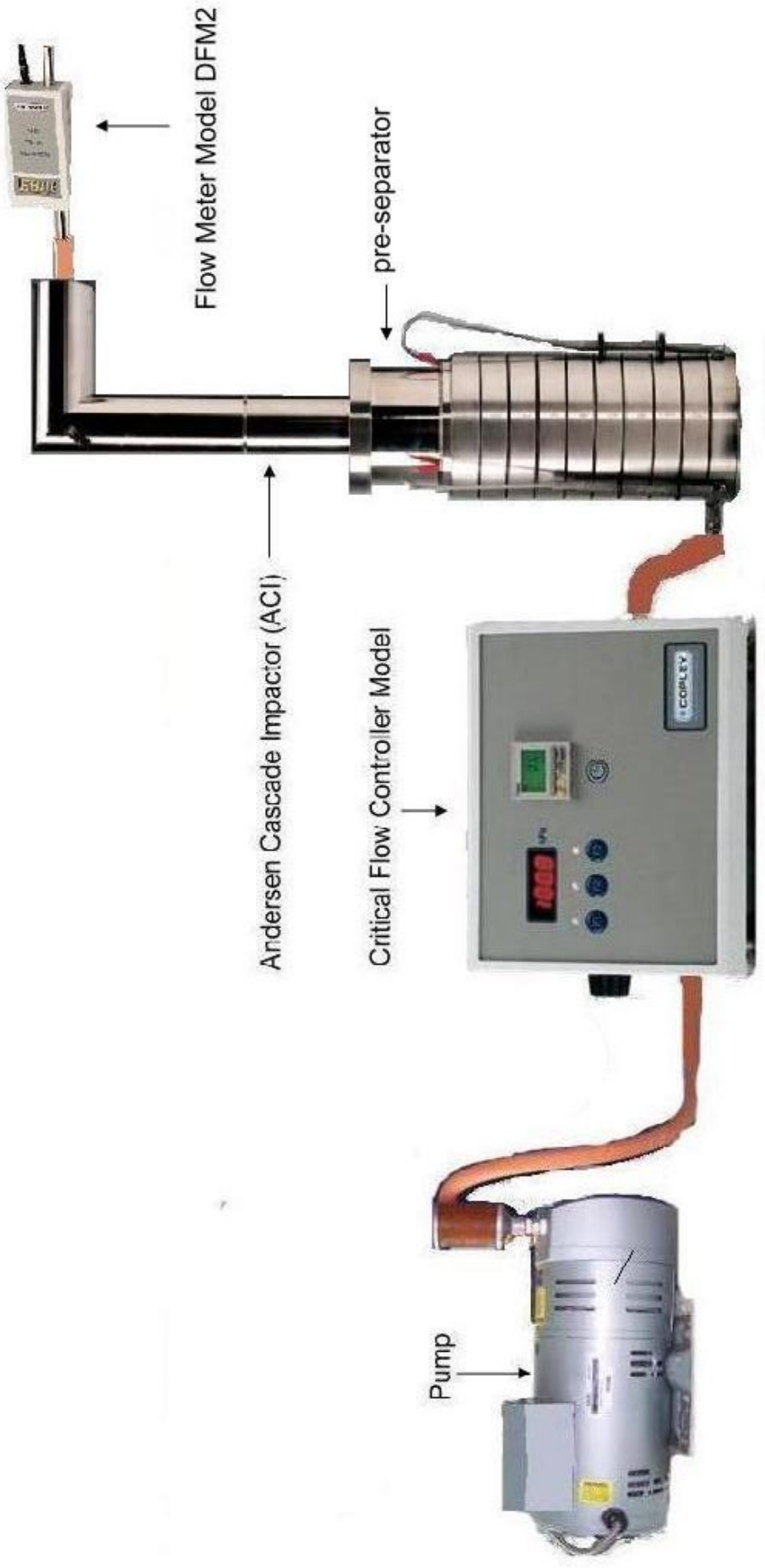

Figure 3.1 The setting of ACI, Critical Flow Controller, and the vacuum pump.

The pre-separator was assembled as follows: 15 ml of washing solution was added to the central cup of the pre-separator base Figure 3.3. Next the pre-separator body was placed on top of the pre-separator base and the two catches were closed. The induction port was then connected to the pre-separator Figure 3.2 a suitable mouthpiece adapter was connected from this point onwards the assembly is similar to previous procedure in section 3.3.2.1

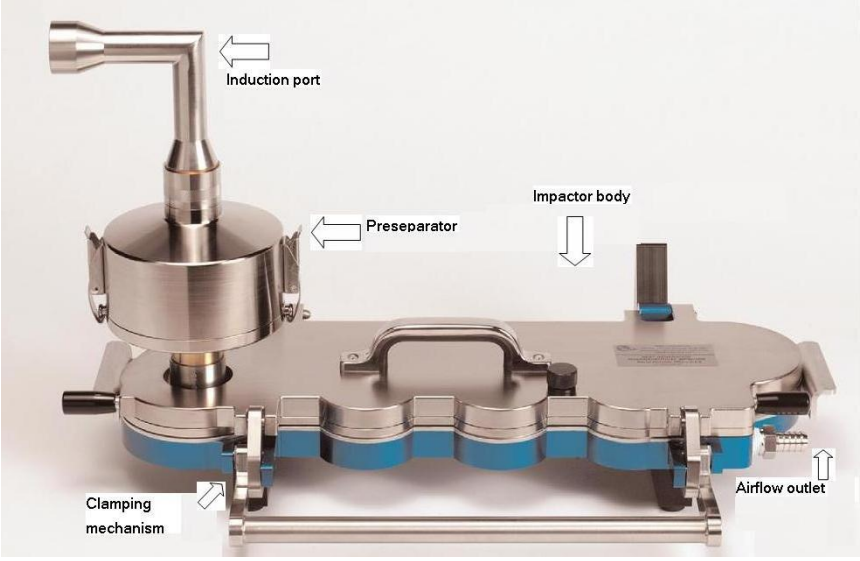

Figure 3.2 Next generation with pre-separator for dry powder particles analysis Source: (Copley, 2007)

Table 3.3 Washing volume of 70% acetonitrile for NGI stages.

Stage	Volume
Induction port	20 ml
Pre-separator	10 ml
Stage 1	10 ml
Stage 2	10 ml
Stage 3	10 ml
Stage 4	10 ml
Stage 5	10 ml
Stage 6	10 ml
Stage 7	5 ml
MOC	5 ml

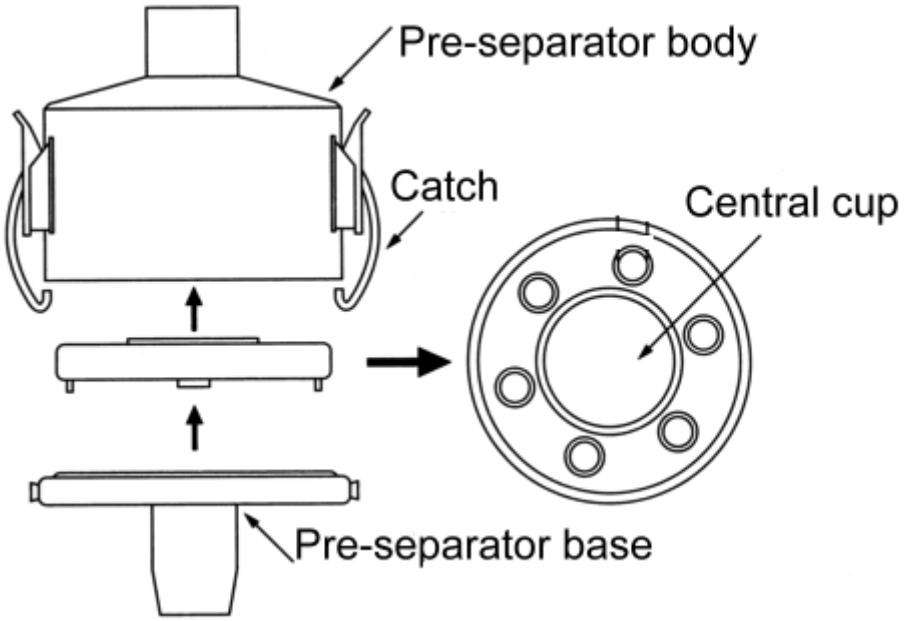

Figure 3.3 Next generation pre-separator configuration Source (USP, 2005)

3.1.3 High-performance liquid chromatography (HPLC) analysis

The amount of drug deposited in each stage was measured using the HPLC method of analysis which was developed and explained in section 2.5,

3.1.4 Fine particle analysis

All the aerodynamic calculation were conducted using the Copley software (CITDAS version 2)

The measured parameters were

- Total Dose Per Shot (TDPS) or ∑A which is the total mass of drug delivered from the mouthpiece of the inhaler into apparatus (USP, 2005).
- Fine Particles Dose (FPD) or R is the total mass of drug found on the stages of the apparatus below a defined size e.g 5 µm and the filter.
- Fine Particle Fraction (FPF) is calculated by the following formula (USP, 2005)

$$FPF = \frac{R}{\sum A} \quad \text{..... Equation 3-1}$$

- In the same way Fine Particle Fraction of Nominal dose is calculated by the following formula

$$FPN = \frac{R}{\text{Nominal dose}} \quad \text{..... Equation 3-2}$$

- Cumulative Percentage of Drug Mass less than Stated Aerodynamic Diameter is calculated as shown in Table 3.4

- Throat deposition is summation of pre-separator and induction port.
- to calculate the Geometric Standard Deviation (GSD) the percentage of mass less than stated aerodynamic is plotted versus D_{50} diameter on log probability paper. Then the GSD can be calculated using the following equation

$$GSD = \sqrt{\frac{SizeX}{SizeY}} \quad \text{..... Equation 3-3}$$

Where Y equal to cumulative percentage of mass less than 15.87% and X equal to cumulative percentage of mass less than 84.13%.

- The Mass Median Aerodynamic Diameter (MMAD) can be calculated as shown in Figure 3.4. which corresponding to cumulative percentage of mass less than 50%

Table 3.4 Calculation of cumulative percentage of mass less than stated aerodynamic diameter

Stage	Cumulative%
Filter	0
Stage7	A= (Drug Mass deposited on the filter)/I X 100
Stage 6	B= (A + Drug Mass deposited on Stage 7)/I X 100
Stage 5	C= (B + Drug Mass deposited on Stage 6)/I X 100
Stage 4	D= (C + Drug Mass deposited on Stage 5)/I X 100
Stage 3	E= (D + Drug Mass deposited on Stage 4)/I X 100
Stage 2	F= (E + Drug Mass deposited on Stage 3)/I X 100
Stage 1	H= (F + Drug Mass deposited on Stage 2)/I X 100
Stage 0	I = (H + Drug Mass deposited on Stage 1)/I X 100

3.1.5 Statistical analysis

The one-way ANOVA with the Bonferroni effect test was used to compare the aerodynamic particle size characterization of the different flow rate using SPSS V15.0 (SPSS Inc., Chicago, USA).

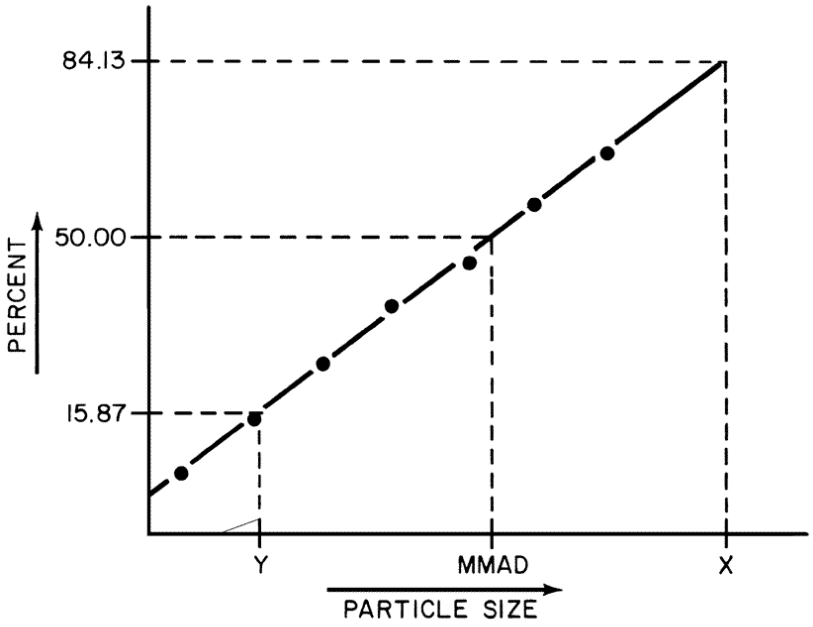

Figure 3.4 Plot of cumulative percentage of mass less than the stated aerodynamic diameter versus aerodynamic diameter. Source (USP, 2005)

RESULT

There are statistically significant differences for formoterol, budesonide R and budesonide S between 28.3L/min and 60 L/min in total emitted dose Tables 3.5 to 3.7. The aerodynamic particles size distribution of formoterol, budesonide R, and budesonide S from the Symbicort® Turbuhaler® device is shown in Table 3.8 to Table 3.19 and Figure 3.5 to Figure 3.11. The comparison of aerodynamic particles size characterization results from the Symbicort® Turbuhaler® device shows that the FPD increases as the flow rate

is increased, this difference was statistically significant $p < 0.001$. Also, there is a statistical difference between flow rate 28.3L/min and 60 L/min in the MMAD. Where it decreases with increasing the flow rate. On the other hand, the effect of flow rate on FPF was statistically significant $p < 0.001$ and the high flow rate increases the FPF. Table 3.8 and Figure 3.6 shows a similarity in the fine particles distribution between the cascade impactors' stages for formoterol, budesonide R, and budesonide S. Furthermore, the STDEV of fine particles distribution reduced as the flow rate was increased which indicates an improvement in dosage form uniformity. On the other hand, as the flow increased, there was a decrease in the amount of formoterol, budesonide R, and budesonide S deposited in the throat induction (port and the pre-separator). Figure 3.11 clearly shows the decrease in the amounts deposited in the throat as the flow increases from 28.3 to 60 L/min. The results of ACI and NGCI at 60 l/min showed an equal result in means of MMAD, FPF, FPFN, FPD, TDPS, GSD, In contrast, there is some differences in STDEV results which showed a higher STDEV with ACI.

The abbreviation used in these tables and figures are as follow:

FPF= Fine Particle Fraction [%]

FPFN = Fine Particle Fraction Nominal dose [%]

TDPS= Total Dose Per Shot [ug]

FPD= Fine Particle Dose [ug]

GSD= Geometric Standard Deviation

MMAD = Mass Median Aerodynamic Diameter.

STDEV= Standard Deviation

AVG= AVERAGE

ACI 28.3 L/min = Andersen Cascade Impactor at flow rate 28.3L/min

ACI 60 L/min = Andersen Cascade Impactor at flow rate 60 L/min

NGI 60 L/min = Next Generation Impactors at 60 L/min

Table 3.5 Mean difference for formoterol (95% confidence interval) compared to formoterol, budesonide R and budesonide S at different flow rate, using ACI and NGI. (* p<0.05, ** < 0.01, ***<0.001 otherwise no significant difference)

		Throat Deposition	Emitted Dose	FPD	FPF	MMAD
ACI 28.3 L/min	Formoterol 60A	20.3** 5.9- 34.8	-10.1* -25.5- 5.2	-36.9*** -50.9- -22.9	-33.3*** -43.1- -23.5	1.3*** 0.9 -1.8
	Formoterol 60N	21.8** 8.7- 35.0	-20.0** -34.0- -6.1	-36.9*** -49.8- -24.0	-30.6*** -39.6- -21.7	1.0*** 0.5- 1.4
	BudisonideR28.3A	6.5 -5.6- 18.7	5.6 -7.3- 18.6	0.1 -12.0- 12.2	-1.6 -10.1- 6.9	0.1 -0.3- 0.6
	BudisonideS28.3A	0.9 -11.3- 13.0	-2.3 -15.3- 10.6	-1.8 -13.9- 10.4	-2.8 -11.3- 5.7	0.0 -0.4- 0.4
ACI 60 L/min	Formoterol 28.3A	-20.3** -34.8- -5.9	10.1* -5.2- 25.5	36.9*** 22.9- 50.9	33.3*** 23.5- 43.1	-1.3*** -1.8- -0.9
	Formoterol60N	1.4 -13.4-16.4	-9.9 -25.7- 5.9	0.0 -14.7- 14.6	2.7 -7.6- 12.9	-0.4 -0.8- 0.1
	BudisonideR60A	-2.6 -18.3- 13.1	2.1 -14.6- 18.8	3.5 -12.1- 19.2	2.3 -8.6- 13.3	-0.1 -0.6- 0.5
	BudisonideS60A	3.8 -11.9- 19.6	4.7 -12.0- 21.4	-0.2 -15.9- 15.4	-2.9 -13.8- 8.1	0.0 -0.5- 0.5
NGI 60 L/min	Formoterol 28.3A	-21.8** -35.0- -8.7	20.0** 6.1- 34.0	36.9*** 24.0- 49.8	30.6*** 21.7- 39.6	-1.0*** -1.4- -0.5
	Formoterol60A	-1.4 -16.4- 13.4	9.9 -5.9- 25.7	0.0 -14.6- 14.7	-2.7 -12.9- 7.6	0.4 -0.1- 0.8
	BudisonideR60N	8.4 -5.2- 22.0	12.9 -1.5- 27.4	3.4 -10.2- 16.9	-3.2 -12.7- 6.3	-0.2 -0.6- 0.3
	BudisonideS60N	7.8 -5.9- 21.4	3.5 -11.0- 17.9	-7.9 -21.5- 5.6	-7.3 -16.8- 2.2	-0.2 -0.6- 0.3

Table 3.6 Mean difference for Budisonide R (95% confidence interval) compared to formoterol, budesonide R and budesonide S at different flow rate, using ACI and NGI. (* p<0.05, ** < 0.01, ***<0.001 otherwise no significant difference)

		Throat Deposition	Emitted Dose	FPD	FPF	MMAD
ACI 28.3 L/min	Formoterol 28.3A	-6.5 -18.7- 5.6	-5.6 -18.6- 7.3	-0.1 -12.2- 12.0	1.6 -6.9- 10.1	-0.1 -0.6- 0.3
	BudisonideR60A	11.2 -3.3- 25.6	-13.7* -29.0- 1.7	-33.4*** -47.4- -19.4	-29.4*** -39.2- -19.6	1.1*** 0.7- 1.6
	BudisonideR60N	23.6** 10.5- 36.8	-12.8* -26.7-1.2	-33.6*** -46.5- -20.8	-32.2*** -41.2- -23.3	0.7** 0.2- 1.1
	BudisonideS28.3A	-5.7 -17.8- 6.5	-8.0 -20.9-4.9	-1.9 -14.0- 10.2	-1.2 -9.67- 7.27	-0.1 -0.5- 0.3
ACI 60 L/min	Formoterol60A	2.6 -13.1- 18.3	-2.1 -18.8- 14.6	-3.5 -19.2- 12.1	-2.3 -13.3- 8.6	0.1 -0.5- 0.6
	BudisonideR28.3A	-11.2 -25.6- 3.3	13.7* -1.7- 29.0	33.4*** 19.4- 47.4	29.4*** 19.6- 39.2	-1.1*** -1.6- -0.7
	BudisonideR60N	12.5 -2.4- 27.3	0.9 -14.9- 16.7	-0.2 -14.8- 14.4	-2.9 -13.1- 7.4	-0.5 -1.0- 0.0
	BudisonideS60A	6.4 -9.3- 22.2	2.6 -14.1- 19.3	-3.7 -19.4- 11.9	-5.2 -16.1- 5.8	0.0 -0.5- 0.6
NGI 60 L/min	Formoterol60N	-8.4 -22.0- 5.2	-12.9 -27.4- 1.5	-3.4 -16.9- 10.2	3.2 -6.3- 12.7	0.2 -0.3- 0.6
	BudisonideR28.3A	-23.6** -36.8- -10.5	12.8* -1.2- 26.7	33.6*** 20.8- 46.5	32.2*** 23.3- 41.2	-0.7** -1.1- -0.2
	BudisonideR60A	-12.5 -27.3- 2.4	-0.9 -16.7- 14.9	0.2 -14.4- 14.8	2.9 -7.4- 13.1	0.5 0.0- 1.0
	BudisonideS60N	-0.6 -14.2- 13.0	-9.4 -23.9- 5.0	-11.3 -24.8- 2.3	-4.1 -13.6- 5.4	0.0 -0.4- 0.5

Table 3.7 Mean difference for Budisonide S (95% confidence interval) compared to formoterol, budesonide R and budesonide S at different flow rate, using ACI and NGI. (* p<0.05, ** < 0.01, ***<0.001 otherwise no significant difference)

		Throat Deposition	Emitted Dose	FPD	FPF	MMAD
ACI 28.3 L/min	Formoterol 28.3-A	-0.9 -13.0- 11.3	2.3 -10.6- 15.3	1.8 -10.4- 13.9	2.8 -5.7- 11.3	0.0 -0.4- 0.4
	BudisonideR28.3A	5.7 -6.5- 17.8	8.0 -4.9- 20.9	1.9 -10.2- 14.0	1.2 -7.3- 9.7	0.1 -0.3- 0.5
	BudisonideS60A	23.3** 8.8- 37.8	-3.1* -18.4- 12.3	-35.3*** -49.3- -21.3	-33.4*** -43.1- -23.6	1.3*** 0.8- 1.8
	BudisonideS60N	28.7*** 15.6- 41.9	-14.2* -28.2- -0.3	-43.0*** -55.9- -30.2	-35.1*** -44.1- -26.1	0.8** 0.4- 1.2
ACI 60 L/min	Formoterol60A	-3.8 -19.6- 11.9	-4.7 -21.4- 12.0	0.2 -15.4- 15.9	2.9 -8.1- 13.8	0.0 -0.5- 0.5
	BudisonideR60A	-6.5 -22.2- 9.3	-2.6 -19.3- 14.1	3.7 -11.9- 19.4	5.2 -5.8- 16.1	0.0 -0.6- 0.5
	BudisonideS28.3A	-23.3** -37.8- -8.8	3.1* -12.3- 18.4	35.3*** 21.3- 49.3	33.4*** 23.6- 43.1	-1.3*** -1.8- -0.8
	BudisonideS60N	5.4 -9.5- 20.3	-11.1 -26.9- 4.7	-7.8 -22.4- 6.9	-1.8 -12.0- 8.5	-0.5 -1.0- 0.0
NGI 60 L/min	Formoterol60N	-7.8 -21.4- 5.9	-3.5 -17.9- 11.0	7.9 -5.6- 21.5	7.3 -2.2- 16.8	0.2 -0.3- 0.6
	BudisonideR60N	0.6 -13.0- 14.2	9.4 -5.0- 23.9	11.3 -2.3- 24.8	4.1 -5.4- 13.6	0.0 -0.5- 0.4
	BudisonideS28.3A	-28.7*** -41.9- -15.6	14.2* 0.3- 28.2	43.0*** 30.2- 55.9	35.1*** 26.1- 44.1	-0.8** -1.2- -0.4
	BudisonideS60A	-5.4 -20.3- 9.5	11.1 -4.7- 26.9	7.8 -6.9- 22.4	1.8 -8.5- 12.0	0.5 0.0- 1.0

Table 3.8 A comparison of amount of formoterol, budesonide R and budesonide S using five doses, deposited on each stage of ACI at 28.3 L/min from Symbicort® Turbuhaler® device

Stage	Formoterol		Budesonide R		Budesonide S	
	AVG	STDEV	AVG	STDEV	AVG	STDEV
Induction port [μg]	3.54	0.62	105.23	22.25	112.33	36.64
Pre-separator [μg]	4.72	1.34	143.97	38.50	159.56	61.87
Stage 0 [μg]	0.06	0.05	1.61	2.11	2.82	3.84
Stage 1 [μg]	0.16	0.08	5.25	3.92	5.24	3.85
Stage 2 [μg]	0.31	0.18	13.18	9.28	13.86	9.21
Stage 3 [μg]	0.84	0.54	26.87	11.20	29.89	11.58
Stage 4 [μg]	0.55	0.27	22.86	8.49	25.45	8.64
Stage 5 [μg]	0.44	0.33	15.14	10.76	16.71	11.27
Stage 6 [μg]	0.00	0.00	0.17	0.04	0.22	0.06
Stage 7 [μg]	0.00	0.00	0.17	0.04	0.22	0.06
Filter [μg]	0.00	0.00	0.17	0.04	0.22	0.06
Nominal Dose [μg]	12		400		400	
Percentage [%]	88.5	20	83.6	14.19	91.63	15.02
TDPS [μg]	10.71	2.37	334.62	56.78	370.19	63.47
FPD [μg]	2.13	1.00	70.48	31.72	77.98	32.19
FPFN [%]	17.73	8.36	17.62	7.93	19.49	8.05
FPF [%]	19.16	5.6	20.77	8.03	21.98	10.68
MMAD [um]	3.62	0.14	3.48	0.16	3.61	0.22
GSD	1.43	0.03	1.44	0.08	1.51	0.14

Table 3.9 A comparison of percentage of formoterol, budesonide R and budesonide S using five doses, deposited on each stage of ACI at 28.3 L/min from Symbicort® Turbuhaler® device.

Stage	Formoterol		Budesonide R		Budesonide S	
	AVG	STDEV	AVG	STDEV	AVG	STDEV
Induction port [%]	33.05	5.86	31.45	7.09	30.34	12.00
Pre-separator [%]	44.05	12.71	43.03	12.27	43.10	20.25
Stage 0 [%]	0.54	0.50	0.48	0.67	0.76	1.26
Stage 1 [%]	1.47	0.71	1.57	1.25	1.41	1.26
Stage 2 [%]	2.85	1.74	3.94	2.96	3.74	3.02
Stage 3 [%]	7.88	5.13	8.03	3.57	8.08	3.79
Stage 4 [%]	5.16	2.59	6.83	2.71	6.87	2.83
Stage 5 [%]	4.13	3.08	4.52	3.43	4.51	3.69
Stage 6 [%]	0.03	0.00	0.05	0.01	0.06	0.02
Stage 7 [%]	0.03	0.00	0.05	0.01	0.06	0.02
Filter [%]	0.03	0.00	0.05	0.01	0.06	0.02

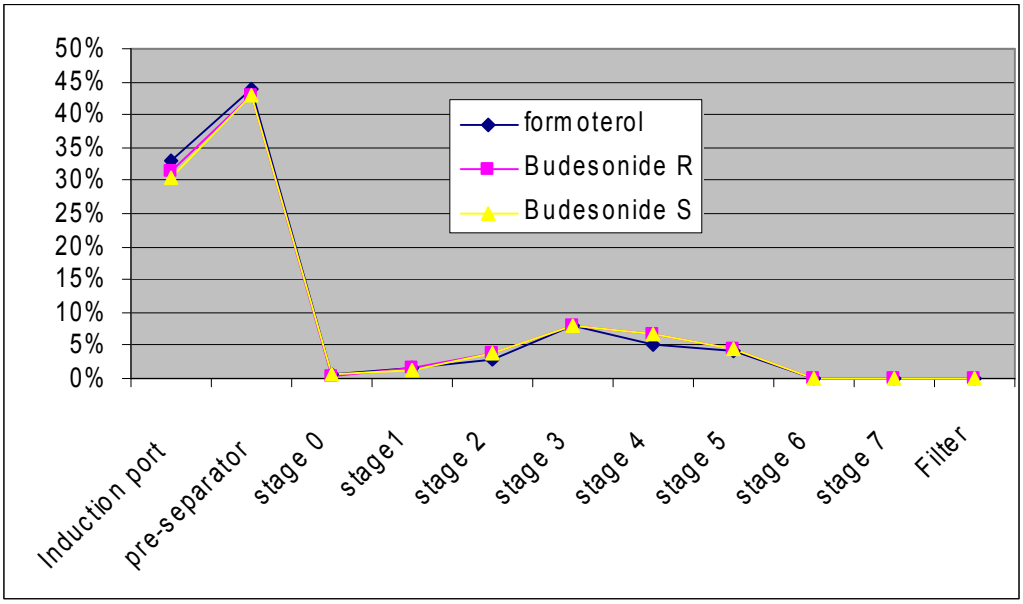

Figure 3.5 A comparison of percentage of formoterol, budesonide R and budesonide S deposited on each stage of ACI, at 28.3 L/min from Symbicort® Turbuhaler® device.

Table 3.10 A comparison of cumulative mass percentages under size for formoterol, budesonide R and budesonide S deposited on each stage of the ACI at 28.3 L/min from Symbicort® Turbuhaler® device

Stage	Formoterol	Budesonide R	Budesonide S
Stage 0 [%}	100.0	100.0	100.0
Stage 1 [%}	100.0	100.0	100.0
Stage 2 [%}	93.20	93.74	94.30
Stage 3 [%}	79.98	78.01	79.20
Stage 4 [%}	43.47	45.95	46.64
Stage 5 [%}	19.55	18.67	18.92
Stage 6 [%}	0.42	0.61	0.71
Stage 7 [%}	0.28	0.41	0.48
Filter [%}	0.14	0.20	0.24

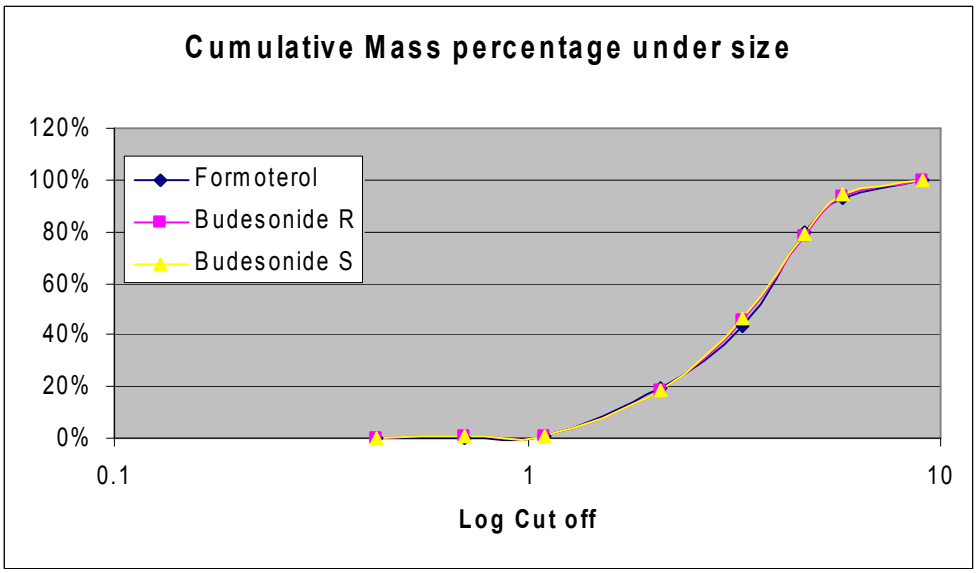

Figure 3.6 A comparison of cumulative mass percentages under size for formoterol, budesonide R and budesonide S deposited on each stage of the ACI at 28.3 L/min from Symbicort® Turbuhaler® device

Table 3.11 A comparison of the amount of formoterol, budesonide R and budesonide S using five doses, deposited on each stage of the ACI at 60 L/min from Symbicort® Turbuhaler® device.

Stage	Formoterol		Budesonide R		Budesonide S	
	AVG	STDEV	AVG	STDEV	AVG	STDEV
Induction port [μg]	4.57	0.62	161.25	25.77	135.52	11.87
Pre-separator [μg]	0.92	0.41	31.97	13.63	31.90	15.76
Stage -1 [μg]	0.11	0.06	1.65	2.55	1.54	2.65
Stage - 0 [μg]	0.27	0.08	6.06	4.00	6.76	4.62
Stage 1 [μg]	0.51	0.30	16.26	10.73	17.88	11.44
Stage 2 [μg]	0.99	0.17	33.81	4.22	35.82	8.49
Stage 3 [μg]	1.81	0.24	60.51	8.22	63.59	15.88
Stage 4 [μg]	1.99	0.99	62.55	30.70	67.20	28.00
Stage 5 [μg]	0.95	0.78	24.09	21.45	28.69	27.69
Stage 6 [μg]	0.30	0.40	6.68	11.14	5.95	10.29
Filter [μg]	0.01	0.01	0.49	0.56	0.01	0.00
Nominal Dose [μg]	12		400		400	
Percentage [%]	103.51	9.36	101.33	17.22	98.71	10.76
TDPS [μg]	12.41	1.12	405.19	68.84	394.85	43.03
FPD [μg]	6.55	1.50	204.27	46.08	219.13	34.87
FPFN [%]	54.58	12.46	51.07	11.52	54.78	8.72
FPF [%]	52.48	8.32	50.15	3.79	55.34	3.90
MMAD [μm]	2.30	0.62	2.35	0.56	2.31	0.63
GSD	1.93	0.23	1.83	0.26	1.86	0.30

Table 3.12 A comparison of the percentage of formoterol, budesonide R and budesonide S using five doses, deposited on each stage of the ACI at 60 L/min from Symbicort® Turbuhaler® device

Stage	Formoterol		Budesonide R		Budesonide S	
	AVG	STDEV	AVG	STDEV	AVG	STDEV
Induction port [%]	34.33	4.68	34.92	5.58	32.06	2.81
Pre-separator [%]	6.88	3.05	6.92	2.95	7.55	3.73
Stage -1 [%]	0.82	0.43	0.36	0.55	0.36	0.63
Stage - 0 [%]	2.05	0.60	1.31	0.87	1.60	1.09
Stage 1 [%]	3.85	2.27	3.52	2.32	4.23	2.71
Stage 2 [%]	7.42	1.28	7.32	0.91	8.47	2.01
Stage 3 [%]	13.62	1.78	13.10	1.78	15.04	3.76
Stage 4 [%]	14.96	7.45	13.54	6.65	15.90	6.62
Stage 5 [%]	7.13	5.83	5.22	4.64	6.79	6.55
Stage 6 [%]	2.23	3.04	1.45	2.41	1.41	2.43
Filter [%]	0.07	0.05	0.11	0.12	0.00	0.00

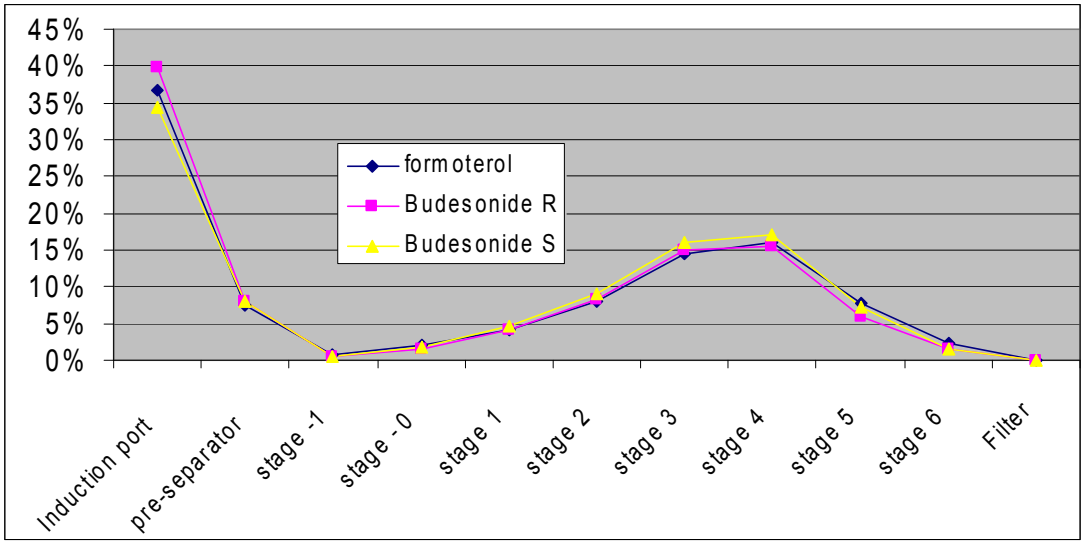

Figure 3.7 A comparison of the percentage of formoterol, budesonide R and budesonide S using five doses, deposited on each stage of the ACI at 60 L/min from Symbicort® Turbuhaler® device

Table 3.13 A comparison of cumulative mass percentages under size for formoterol, budesonide R and budesonide S deposited on each stage of the ACI at 60 L/min from Symbicort® Turbuhaler® device

Stage	Formoterol	Budesonide R	Budesonide S
Stage – 1 [%]	100.00	100.00	100.00
Stage -0 [%]	98.49	99.36	99.43
Stage 1 [%]	94.59	96.67	96.64
Stage 2 [%]	86.38	88.31	88.18
Stage 3 [%]	71.32	71.43	71.68
Stage 4 [%]	43.86	41.44	42.50
Stage 5 [%]	16.28	13.09	13.76
Stage 6 [%]	3.75	2.83	2.21
Filter [%]	0.15	0.28	0.00

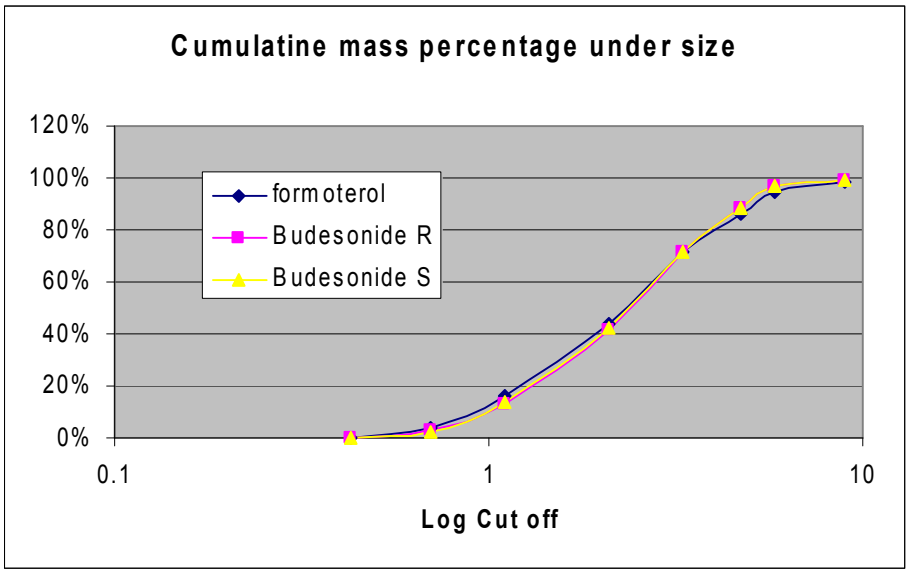

Figure 3.8 A comparison of cumulative mass percentages under size for formoterol, budesonide R and budesonide S deposited on each stage of the ACI at 60 L/min from Symbicort® Turbuhaler® device

Table 3.14 A comparison of the percentage of formoterol, budesonide R and budesonide S using five doses, deposited on each stage of the NGI 60 L/min from Symbicort® Turbuhaler® device

Stage	Formoterol		Budesonide R		Budesonide S	
	AVG	STDEV	AVG	STDEV	AVG	STDEV
Induction port [%]	36.34	6.73	37.46	3.11	35.01	4.29
Pre-separator [%]	4.68	1.4	0.47	0.23	0.13	0.24
Stage 1 [%]	1.87	0.25	0.68	0.51	0.47	0.56
Stage 2 [%]	10.17	1.27	12.41	1.64	12.28	1.53
Stage 3 [%]	15.34	1.93	17.88	1.43	19.18	1.57
Stage 4 [%]	17.9	1.97	20.26	0.63	21.45	1.04
Stage 5 [%]	10.11	1.07	9.47	0.29	10.57	0.83
Stage 6 [%]	3.07	0.17	0.79	0.58	0.92	0.62
Stage 7 [%]	0.5	0.14	0.27	0.19	0	0
MOC [%]	0.03	0.03	0.3	0.19	0	0

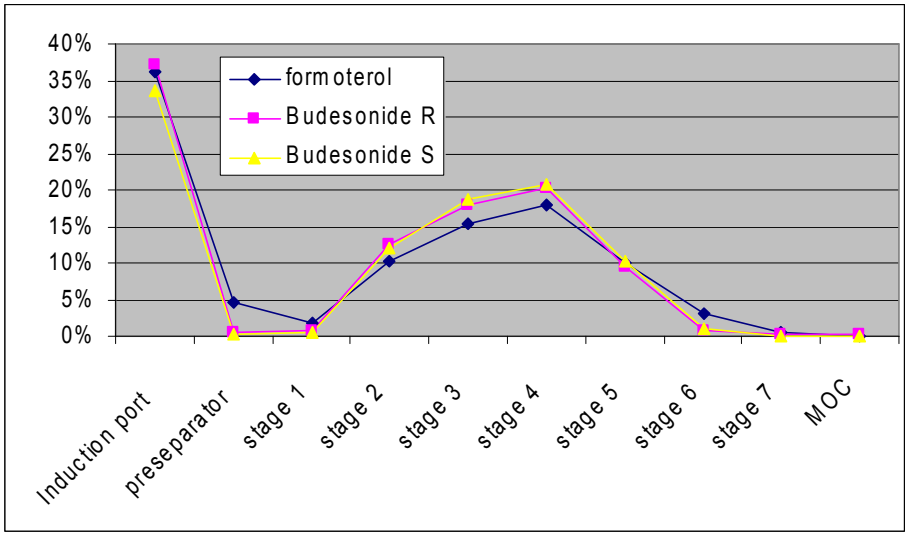

Figure 3.9 A comparison of the percentage of formoterol, budesonide R and budesonide S using five doses, deposited on each stage of the NGI at 60 L/min from Symbicort® Turbuhaler® device

Table 3.15 A comparison of the amount of formoterol, budesonide R and budesonide S using five doses, deposited on each stage of the NGI at 60 L/min from Symbicort® Turbuhaler® device

Stage	Formoterol		Budesonide R		Budesonide S	
	AVG	STDEV	AVG	STDEV	AVG	STDEV
Induction port [μg]	4.75	1.00	143.3	22.22	146.8	5.98
Pre-separator [μg]	0.60	0.13	1.72	0.56	0.60	1.15
Stage 1 [μg]	0.25	0.06	2.90	2.58	2.14	2.66
Stage 2 [μg]	1.35	0.32	48.59	14.82	52.50	11.69
Stage 3 [μg]	2.03	0.46	69.50	17.92	81.65	15.18
Stage 4 [μg]	2.35	0.44	78.03	14.89	91.10	13.71
Stage 5 [μg]	1.32	0.21	36.46	6.71	44.66	4.83
Stage 6 [μg]	0.40	0.06	3.36	2.82	4.08	2.82
Stage 7 [μg]	0.07	0.02	0.93	0.49	0.01	0.00
MOC [μg]	0.00	0.00	1.05	0.45	0.01	0.00
Nominal Dose	12		400		400	
Percentage [%]	109.4	13.84	96.46	19.11	105.9	11.63
TDPS [μg]	13.13	1.66	385.8	76.43	437.5	29.32
FPD [μg]	6.56	1.27	205.0	44.7	250.2	25.8
FPFN [%]	54.63	10.61	51.27	11.19	62.56	6.45
FPF[%]	49.80	5.36	53.03	1.80	57.10	2.10
MMAD [μm]	2.65	0.09	2.82	0.13	2.80	0.07
GSD	1.88	0.02	1.74	0.04	1.72	0.05

Table 3.16 A comparison of cumulative mass percentages under size for formoterol, budesonide R and budesonide S deposited on each stage of the NGI at 60 L/min from Symbicort® Turbuhaler® device

Stage	Formoterol	Budesonide R	Budesonide S
Stage 1 [%]	100.0	100.0	100.0
Stage 2 [%]	96.81	98.92	99.31
Stage 3 [%]	79.57	78.98	80.42
Stage 4 [%]	53.59	50.20	50.86
Stage 5 [%]	23.25	17.50	17.76
Stage 6 [%]	6.10	2.19	1.39
Stage 7 [%]	0.88	0.93	0.01
MOC [%]	0.04	0.49	0.00

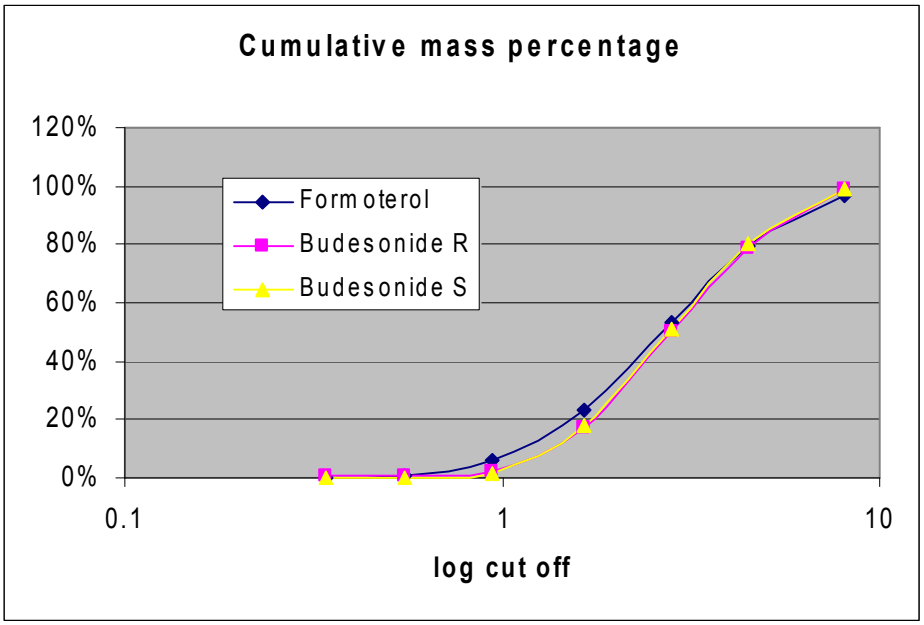

Figure 3.10 A comparison of cumulative mass percentages under size for formoterol, budesonide R and budesonide S deposited on each stage of the NGI at 60 L/min from Symbicort® Turbuhaler® device

Table 3.17 Percentage of formoterol using five doses, deposited on each stage of ACI at two different flow rate 28.3 L/min and 60 L/min and NGI at 60L/min. from Symbicort® Turbuhaler® device diveded by number of doses

	ACI 28.3		ACI 60		NGI 60	
	AVI	STDEV	AVI	STDEV	AVI	STDEV
Induction port [%]	33.48	5.86	34.33	4.68	36.34	6.73
Pre-separator [%]	44.63	12.71	6.88	3.05	4.68	1.4
Percentage [%]	88.58	20	103.5	9.36	109.4	13.84
TDPS [μg]	10.71	2.37	12.41	1.12	13.13	1.66
FPD [μg]	2.13	1	6.55	1.5	6.56	1.27
FPFN [%]	17.73	8.36	54.58	12.46	54.63	10.61
FPF [%]	19.16	5.6	52.48	8.32	49.8	5.36
MMAD [μm]	3.62	0.14	2.3	0.62	2.65	0.09
GSD	1.43	0.03	1.93	0.23	1.88	0.02
Flow Rate [L/min]	28.3		60		60	

Table 3.18 Amount (ug) of budesonide R using five doses, deposited on each stage of the ACI at two different flow rate 28.3 L/min and 60 L/min and NGI at 60L/min. from Symbicort® Turbuhaler® device

	ACI 28.3		ACI 60		NGI 60	
	AVG	STDEV	AVG	STDEV	AVG	STDEV
Induction port [%]	33.54	7.09	34.92	5.58	36.34	6.73
Pre-separator [%]	45.90	12.27	6.92	2.95	4.68	1.40
Percentage [%]	78.42	14.19	101.33	17.22	96.46	19.11
TDPS [μg]	334.62	56.78	405.19	68.84	385.84	76.43
FPD [μg]	70.48	31.72	204.27	46.08	205.06	44.7
FPFN [%]	17.62	7.93	51.07	11.52	51.27	11.19
FPF [%]	20.77	8.03	50.15	3.79	53.03	1.80
MMAD [μm]	3.48	0.16	2.35	0.56	2.82	0.13
GSD	1.44	0.08	1.83	0.26	1.74	0.04
Flow Rate [L/min]	28.3		60		60	

Table 3.19 Amount (ug) of budesonide S using five doses, deposited on each stage of the Andersons Cascade impactor at two different flow rate 28.3 L/min and 60 L/min and Next generation Cascade impactor at 60L/min. from Symbicort® Turbuhaler® device diveded by number of doses

	ACI 28.3		ACI 60		NGI 60	
	AVG	STDEV	AVG	STDEV	AVI	STDEV
Induction port [%]	36.77	12.00	32.06	2.81	35.01	4.29
Pre-separator [%]	52.23	20.25	7.55	3.73	0.13	0.24
Nominal Dose	400	0.00	400	00	400	0.00
Percentage [%]	76.37	15.02	98.71	10.76	105.90	11.63
TDPS [μg]	305.48	63.47	394.85	43.03	437.50	29.32
FPD [μg]	112.40	32.19	219.13	34.87	250.23	25.8
FPFN [%]	28.10	8.05	54.78	8.72	62.56	6.45
FPF [%]	36.79	10.68	55.34	3.90	57.10	2.10
MMAD [um]	3.46	0.22	2.31	0.63	2.80	0.07
GSD	1.55	0.14	1.86	0.30	1.72	0.05
Flow Rate [L/min]	28.3		60		60	

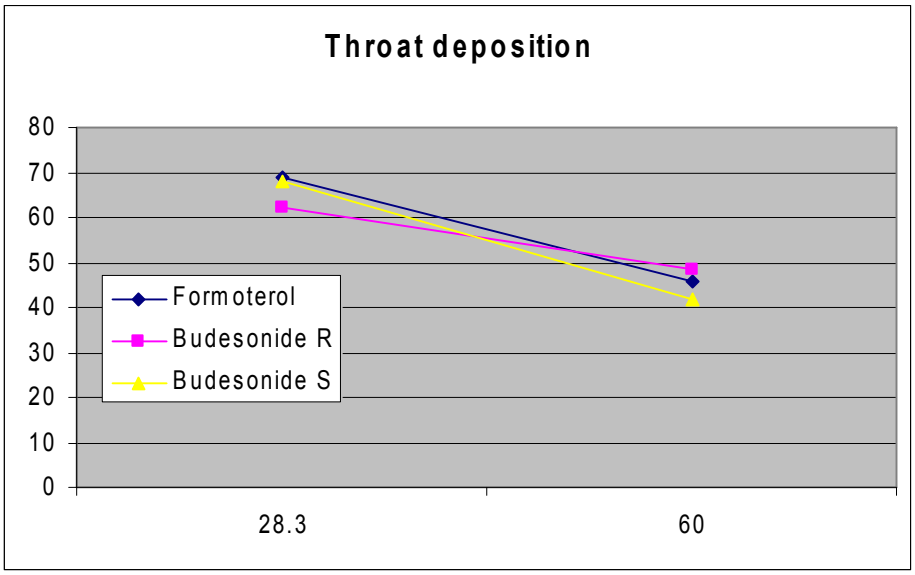

Figure 3.11 Percentage of formoterol, budesonide R, and budesonide S using five doses, deposited on pre-separator stage + induction port (throat deposition) of the ACI at 28.3L/min and 60 L/min from Symbicort® Turbuhaler® device.

DISCUSSION

The influence of flow rate on drug delivery via the Turbuhaler® has been investigated in this study. The results showed that a direct relationship exists between flow rate through the Turbuhaler® and the deposition on the stages. The literatures suggested similar data were reported for terbutaline sulphate in an *in-vivo* study (Newman et al., 1991). Equally *in-vitro* results suggested that a high flow rate through the Turbuhaler® eases deaggregation of drug particles, it reduces particle impaction in the oropharynx, and therefore enhances lung deposition (Jaegfeldt et al., 1987). Other studies on dry powder inhalers when operated at 28 L/min revealed lower value for drug lung deposition. For instance the value for Spinhaler® was 12% (Auty et al., 1987), and for Rotahaler® and Diskhaler® were close to 10% (Vidgren et al., 1988, Biddiscombe M, 1991, Biddiscombe et al., 1993).

Tarsin and co-workers (2006) demonstrate that adult patients with severe asthma achieved a high inspiratory flow rate through the Turbuhaler®. On the other hand it has been reported that, the most preferred inhalation rate for Turbhaler was 60 L/min (Borgstrom et al., 1996). In addition, Engel co-workers (1989) measured the flow through the Turbuhaler device for a variety of patients with different severities of asthma using the FEV,% of predicted as the severity marker they found the mean peak inspiratory flow rate was 59 L/min with a range from 25 to 93 L/min.

The results of the MMAD and FPF showed a decrease in value as the flow was increased. The Symicort® formulation consists of formoterol, budesonide and lactose monohydrate as carrier (SYMBICORT®_Product_Information, 2008). The carrier plays an important factor for the fine particle size

dispersion. It has been recognized earlier that separation of drugs and carrier particles occurs easer when the carrier crystals are smaller (Engel et al., 1992). Engel and co-workers (1992) examined a mixture of small drug crystals and large carrier particles, and they found the drug particles larger than 5 μm are separated at flows of 60 L/min while particles in the size range 5 – 7 μm needed a higher inhalation flow to be separated. As a consequence, the efficiency of the penetration and deposition within the airways is dependant upon both the inhalation flow rate and the aerodynamic size distribution of the inhaled aerosol. At a specific flow rate, the higher pressure drop devices such as Turbuhaler produced a higher FPF compared with the lower pressure drop devices that used capsule reservoirs. The high specific resistance devices would be expected to generate higher turbulence, and thus the high turbulence will generate a higher FPF (Engel et al., 1990).

For a patient reaching high inhalation flows it will be more likely that lung deposition will be increased and hypothetically more doses would be deposited in the central zone of the lungs.

Amounts of budesonide and formoterol emitted from the same dose were similar. The inter-inhaler variability for the combination product is similar to that previously shown in a study (Tarsin et al., 2004). Nevertheless, Zanen and co-workers (1992) demonstrated that the high availability of salbutamol mass due to increasing the flow rate was not expressed as a stronger bronchodilator. Also they concluded that the impactor is able to detect minor differences but these are too small to make clinical differences.

Figure 1.11 shows that at a 60 L/min flow rate the particles deposition in the throat the pre-separator and the induction port) has reduced compared to a

flow rate of 28.3L/min. An explanation for this change can be linked to the change in flow rate. Since the high flow rate enhances particle de-aggregation and reduces the MMAD as result less large particles will be available to deposit in the pre-separator (Jaegfeldt et al., 1987).

The cumulative mass percentages under size 3 μm have increased for formoterol and two epimers of budesonide. Zanen and co-workers (1996) conducted a study to determine the optimal particle size for a bronchodilator and their results demonstrated that the 3 μm has an optimum clinical effect. The study also showed that particles having a diameter greater than 5 μm have a tendency to either deposit in the throat or the mouth and therefore, they show significantly lower clinical effect.

CONCLUSION

This chapter has helped in the understanding of the effect of flow on the particle size deposition into various regions of the lung. *In-vitro* flow–dependency for particle deposition among cascade impactors has been demonstrated for a combination of budesonide and formoterol. The effect of this was more obvious for the FPD and the throat deposition. From the data obtained it can be expected that at a lower flow rate the drug particles might be deposited on the oropharyngeal. In addition the low fine particle dose indicated that lung deposition would be low.

Since, most of the DPIs such as the Turbuhaler®, devices depend on the patient's inspiratory influence to emit and deaggregate the drug, device design should minimize patient factors such as flow rate effects, environmental effects, and complexity in operation this will ensure that the patient receive a safe and efficacious dose. The design of different dry powder inhaler devices

results in different resistances. A higher air velocity is more likely to induce turbulent air than a low velocity. The higher the turbulence generated by the device, the greater the FPF that is likely to be obtained. It is a principle in the formulation of a dry powder that the device should give a high FPF of drug whilst the carrier such as lactose, in the formulation should remain only in the upper airways.

The flow-dependent particle deposition results emphasize the need for the Pharmacopoeias to use a variety of inhalation flow rates for in-*vitro* tests rather than one that is determined according to the resistance of the dry powder inhaler.

CHAPTER 4

Performance of a small-volume valved holding chamber and small spacer with several beclomethasone brand-name preparations delivered by pressurised metered dose inhalers.

4 PERFORMANCE OF SMALL-VOLUME VALVED HOLDING CHAMBER AND SMALL SPACER WITH SEVERAL BECLOMETHASONE BRAND-NAME PREPARATIONS DELIVERED BY PRESSURISED METERED DOSE INHALERS.

OBJECTIVE

The main objective of this study is to examine the dose emission of different brand names of beclomethasone formulations alone and attached to spacers. Many parameters have been compared including washed versus unwashed and the number of actuated doses. A secondary aim is to examine the hypothesis of whether the result of a specific spacer with a given drug/ brand name can be extrapolated to other pMDIs or brand names for the same drug.

INTRODUCTION

As shown in the literatures review pMDIs are a convenient way of administering medication which includes bronchodilators and corticosteroids, for patients with asthma and COPD. As indicated they emit an aerosol at high velocity and to be used properly they require coordination of inhalation and pMDI actuation. But, even with an optimum technique, only < 15% of the emitted dose regularly reaches the airways. Spacer devices were introduced to try to improve the efficacy of inhaled therapy with pMDIs by decreasing the need for coordination between actuation and inhalation and by allowing deceleration and evaporation of propellant, so decreasing oropharyngeal deposition of therapy. In operation however, part of the inhaled drug is lost within the spacer. The major cause of the loss is impaction due to inertia, sedimentation due to reduced speed of the aerosol particles and adsorption due to electrical charge Figure 4.1. The loss by impaction occurs immediately after actuation, and loss due to sedimentation and adsorption is time-

dependent (Bisgaard et al., 2002). Many different spacer devices are currently available, some designed to fit with one particular product, while others are intended for use with a variety of pMDIs.

During these early devices, CFCs have traditionally served as the propellant of choice for use with pMDIs. But as discussed, they deplete the Ozone Layer as initially noted by Molina and Rowland (Molina et al., 1974). This discoverery and the introduction of legislation prompted a change.

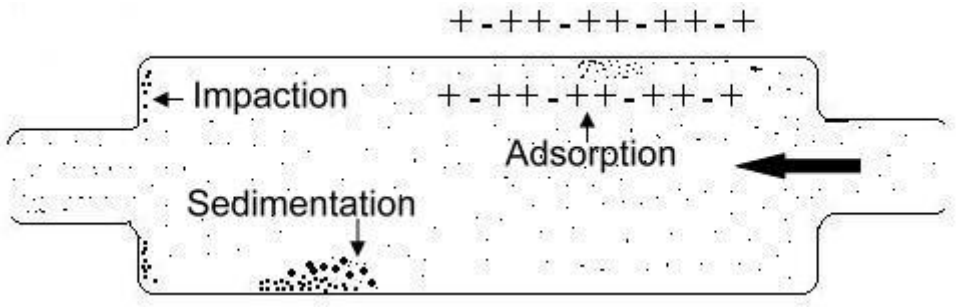

Figure 4.1 Mechanism of aerosol loss within a spacer.

Example was DPIs which were recognised as a suitable alternative; however, not all inhaled drug were available in DPIs. Furthermore, they are not always suitable for all patients, for example, those with low inspiratory flow rate (young, asthmatic, elderly and COPD patients) and low cognitive capability patients. Also DPIs may not be suitable in certain humid climates. In some situations, their extra expense would also deprive many of necessary therapy.

As the same time as the development of DPIs alternative propellants were being investigated in pMDIs, HFAs were identified as suitable, non-ozone-depleting alternatives (Terzano, 2001). In addition, HFA134a was approved by the Committee for Proprietary Medicinal Products (CPMP) under the European Medicines Evaluation Agency (EMEA), As a result of the introduction of these new propellants, many formulation changes were

needed. And the incorporation of co-solvents such as ethanol, was used in either suspension or solution formulations (Cripps et al., 2000). This reformulation however led to discernible changes in some properties of the final products, which included extra-pulmonary deposition, taste and/ or lung deposition (Bisgaard et al., 2002).

In this work the following formulations are examined:

- Qvar® is a CFC-free pMDI designed for oral inhalation. Each unit contains a solution of beclomethasone dipropionate in HFA-134a (1,1,1,2 tetrafluoroethane) propellant and ethanol. In operation the Qvar should be actuated twice prior to use of the first dose from a new canister or when the inhaler has not been used for more than 10 days (Qavr®_Product-information, 2006).
- Beclazone® is a CFC formulation, which provides beclomethasone in a suspension form. The Beclazone list of excipients comprises oleic acid, dichlorodifluoromethane and trichlorofluoromethane (Beclazone_Product-information, 2004).
- The third inhaler used in this work, Clenil® also uses HFA 134a as the propellant. The formulation is a solution as opposed to the suspension formulations which were formerly used in pMDIs containing CFC. This pMDI is formulated from glycerol as non-volatile co-solvent, anhydrous ethanol as co-solvent and HFA-134 as propellant (CLENIL®_Product-information, 2007b).
- Becloforte ® and Becotide ® are CFC formulations, with the same formulation consisting of oleic acid, dichlorodifluoromethane and

trichlorofluoromethane (Becotide™_Product-information, 2007, Becloforte_Product-information, 2003).

Table 4.1 summarises the list of excipients of inhalers used in the experiments. And the spacers are indicated below:

- AeroChamber MAX® (AMAX) is VHC. The volume of AMAX is 198 mL. It is manufactured from a shatter-resistant, clear, anti-static polymer blend. The chamber has been completely redesigned for use with HFAs formulations. Furthermore, it is a universal pMDI VHC. It incorporates a Flow-Vu™ the Inspiratory Flow Indicator to provide the caregiver with reassurance of medication delivery to the lungs. Also it has a one-way, low resistance duckbill valve system (Aerochamber_Max_Product-Monograph, 2006).
- In contrast with the AMAX, the Aerochamber PLUS® VHC (APLUS) is prone to static and the volume is 149 mL. But it can be used with various pMDIs (Aerochamber_Plus_Product-Monograph, 2005).
- The Optimizer® is a spacer with 50 mL volume. It comprises a plastic tube with a cross section of 2.5 x 3.5 cm. and has an overall length of 10 cm (Hardy et al., 1996).

METHODOLOGY

4.1.1 Instrumentation

4.1.1.1 Equipment and inhalation device

The equipment used for the dose emission study is described in section 2.1 2.2.

The analytical HPLC method is detailed in section 2.6.

Table 4.1 List of excipients.

Inhaler	CFC	Alcohol content / actuation	Excipients
Becotide	Yes[a]	Nil[b]	Oleic acid, dichlorodifluoromethane trichlorofluoromethane[c]
Becloforte	Yes[a]	Nil[b]	Oleic acid, dichlorodifluoromethane trichlorofluoromethane[d]
Beclazone Easi-Breathe	Yes[a]	Nil[b]	Oleic acid, dichlorodifluoromethane trichlorofluoromethane[e]
Beclazone pMDI	Yes[a]	Nil[b]	Oleic acid, dichlorodifluoromethane trichlorofluoromethane[e]
Qvar pMDI	No[a]	8.35 mg[b]	HFA-134a, ethanol[f]
Qvar Easi-Breathe	No[a]	8.35 mg[b]	HFA-134a, ethanol[f]
Qvar pMDI 50 µg	No[a]	8.35 mg[b]	HFA-134a, ethanol[f]
Clenil modulite	No[a]	9mg[b]	Glycerol, ethanol anhydrous HFA-134[g]

a- (BNF, 2008). b- (Alrasbi et al., 2008), c- (Becotide™_Product-information, 2007), d- (Becloforte_Product-information, 2003), e- (Beclazone_Product-information, 2004), f- (Qavr®_Product-information, 2006),g- (CLENIL®_Product-information, 2007b)

4.1.1.2 Instrumentation set up

The dose emission method described in the British Pharmacopeia (BP) for pMDIs was used. The initial work was to prepare the sampling apparatus for pMDIs Figure 4-2. All parts of the sampling apparatus for pMDIs were washed with acetone and dried and the apparatus was then assembled as described in the manufacture's manual, which incorporates an after filter to capture any particles which would otherwise escape from the apparatus.

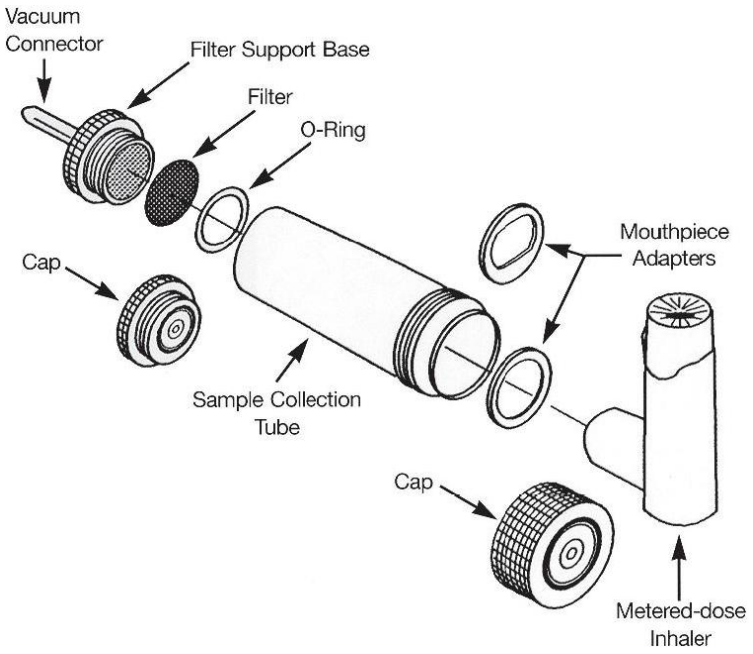

Figure 4.2 Sampling Apparatus for pMDIs Source: (Copley, 2007).

Figure 4.3 illustrates the positioning of the sampling apparatus for pMDIs, the critical flow controller and the vacuum pump. The mouthpiece adapter was attached to the end of the sampling apparatus for the pMDIs/spacer. The flow control valve was adjusted to achieve a steady flow through the system at the required rate, 28.3L/min (±5%) which was measured by an electronic digital flow meter Model (DFM). According to the pharmacopeia method, 4 L of air was drawn through the inhaler for each determination and the absolute pressure ratio P3/P2 < 0.5 was confirmed.

Each pMDI/spacer was prepared according to the patient leaflet instructions (see appendix A) and was actuated into the collecting tube by opening the valve if the device was breath-actuated, otherwise it was operated by pressing down on the inhaler to release its contents (n=10). When 2 doses were required the patient leaflet instructions were followed (see appendix A).

Figure 4.3 The setting of Sampling Apparatus, Critical Flow Controller and vacuum pump

The time 8.4 sec (±5%) was calculated according to equation 4-1.

$$T = \frac{60\,\text{sec} \times X}{Q} \quad \text{..... Equation 4-1}$$

where T = Time duration consistent for withdrawal of X litres of air from the inhaler

Q = Flow rate required

X = Volume L to be drawn through inhaler

The mean content of drug per actuation was tested at different points between the first and final actuation. Randomised sampling tables were used for studying the dose emission over the entire set of inhalers.

Determinations were made for each pMDI and for attached to each of the three different spacers. The spacers were the Optimizer® spacer, the AMAX and the APLUS. Table 4.2 summarise the experiments. In these experiments, the Becotide actuator was washed with water and was compared with unwashed Becotide.

Once used the apparatus was dismantled carefully to avoid any loss of material. And the emitted dose was washed from the collection tube/spacer into 50 mls of a washing solution (acetonitrile: water 70:30, v/v).

Table 4.2 List of dose emission experiments conducted

Experiment n=10
Beclazone 100 µg 2 doses Easi-Breathe®
Beclazone100 µg 2 doses AMAX
Beclazone100 µg 2 doses APLUS
Beclazone 250 µg 1 dose Easi-Breathe® Optimiser®
Beclazone 250 µg 2 doses Easi-Breathe®
Beclazone 250 µg 2 doses Easi-Breathe® Optimiser
Becloforte 1 dose
Becloforte 1 dose AMAX
Becloforte 1 dose APLUS
Becloforte 2 doses
Becloforte 2 doses APLUS
Becotide 100 µg 2 doses unwashed
Becotide 100 µg 2 doses washed
Becotide100 µg 2 doses AMAX
Becotide 100 µg 2 doses Optimiser
Clenil 100 µg 2 doses
Clenil 100 µg 2 doses AMAX
Clenil 100 µg 2 doses APLUS
Clenil 250 µg 1 dose
Clenil 250 µg 1 dose AMAX
Clenil 250 µg 1 dose APLUS
Qvar 50 µg 1 dose Aerosol
Qvar 50 µg 1 dose Aerosol APLUS
Qvar 50 µg 2 doses Aerosol
Qvar 50 µg 2 doses Aerosol AMAX
Qvar 50 µg 2 doses Aerosol APLUS
Qvar 100 µg 1 dose Aerosol Optimiser
Qvar 100 µg 2 doses Aerosol Optimiser
Qvar 100 µg 2 doses Easi-Breathe®
Qvar 100 µg 1 dose Easi-Breathe AMAX
Qvar 100 µg 1 dose Easi-Breathe APLUS
Qvar 100 µg 1 dose Easi-Breathe Optimizer
Qvar 100 µg 1 dose Easi-Breathe

Both the collecting tube and spacer content is reported separately. The drug under test was extracted from the filter into the washing solution.

In order to ensure complete extraction, the filter was sonicated for three minutes in the washing solution. Also, the washing solution from the filter was further filtered through a 0.45 µm filter in order to remove any unwanted

particles which might block the HPLC system. The amount of drug was determined by HPLC using the previously validated method described in section 2.6.

STATISTICAL ANALYSIS

One-way ANOVA with Bonferroni effect test was used to compare the emitted dose with different brands using SPSS V15.0 (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

4.1.2 Dosage uniformity

Table 4.3 shows the measured reproducibility of dose emissions (dosage uniformity) from each of the inhalers and average percentage of label claim emitted from each inhaler. For comparative purposes, the emitted percentage was calculated as the amount of drug extracted from the sampling apparatus expressed as a percentage of the product label claim. On the other hand, when the spacers were compared together, the amount of delivered drug was expressed as a percentage of the product of the emitted dose. However, when the performance of the spacer with drug alone was compared, the amount of delivered drug was expressed as a percentage of the product nominal dose.

The variability of dose emissions from the pMDIs was found to be relatively high, particularly those with the CFC formulations, both within and between devices. The STDEV associated with the emitted doses from CFC-free formulations devices were significantly lower e.g. Qvar ®. Bisgard and co-workers (2002) reported that the introduction of the CFC-free formulation has led to improve dosage uniformity from first to last with the new devices.

Table 4.3 Average and ±STDEV of total emitted dose % (nominal dose) from different formulations of beclomethasone at 28.3 L/min.

Experiment (n=10)	Delivered drug (%)	STDEV (%)
Qvar 50 µg 2 doses	86.26	3.82
Qvar 100 µg Easi-Breathe® 1 dose	101.33	4.83
Qvar 50 µg 1 dose	84.67	5.37
Beclazone 100 µg Easi-Breathe® 2 doses	100.61	5.61
Clenil 100 µg 2 doses	97.42	7.73
Qvar 100 µg Easi-Breathe® 2 doses	100.86	8.33
Clenil 250 µg 1 dose	95.73	9.49
Becloforte 2 doses	100.58	11.23
Beclazone 250 µg Easi-Breathe® 2 doses	93.54	15.42
Becloforte 1 dose	101.02	18.95
Becotide 100 µg 2 doses washed	106.66	22.00
Becotide 100 µg 2 doses unwashed	116.53	27.01

STDEV= Standard Deviation

Cripps co-workers (2000) showed consistent performance for HFA134a formulated pMDIs, and the overall STDEV per actuation was within 10%. Moreover, CFC inhalers are suspensions and usually, suspension formulations are sensitive to the effect of separation of the suspended drug within the valve on standing due to sedimentation or creaming. As a result the dosing characteristic of the inhaler may be affected.

There is a significant difference between the unwashed and washed average emitted dose for Becotide ($p < 0.05$) and there is also a difference between the STDEV for unwashed and washed Becotide. Failure to wash the mouthpiece can lead to inconsistent dose and aerosol particle size (Terzano, 2001). Furthermore, Berlnski (2001) reported that actuators should be cleaned to avoid build up of drug in them.

4.1.3 Effect of number of doses

One of the factors which has been tested is the effect of number of doses on the total emitted dose. Since it is a common practice to give a patient two doses and assume that the two doses deliver double the amount of drug compared to one dose. But, this may not be the case in practice, if there is build up of drug in the delivery unit or a variation in delivery from the inhaler.

Table 4.4 Effect of number of doses (95% confidence interval) on percentage of delivered dose (% nominal dose).

Experiment (n=10)	Delivered dose [%]	STDEV	Mean Difference
Qvar 50 µg 1 dose	84.67	5.37	1.6- 6.6 -9.8-
Qvar 50 µg 2 doses	86.26	3.82	
Qvar 100 µg Easi-Breathe® 1 dose	101.33	4.83	0.5 8.7 -7.8-
Qvar 100 µg Easi-Breathe® 2 doses	100.86	8.33	
Becloforte 1 dose	101.02	18.95	0.4 8.7 -7.8-
Becloforte 2 doses	100.58	11.23	
Qvar 50 µg 1 dose aerosol APLUS	54.52	2.8	-6.6 -14.8-1.6
Qvar 50 µg 2 doses aerosol APLUS	61.17	8.5	
Qvar 1 dose Easi-Breathe® 100 µg APLUS	62.62	10.20	-1.5 -9.7- 6.8[a]
Qvar 100 µg aerosol 1 dose optimiser	57.48	4.9	-10.8 -19.0 -2.6
Qvar 100 µg aerosol 2 doses optimiser	68.29	12.34	
Beclazone Easi-Breathe® 250 µg 1 dose optimiser	50.62	13.2	3.1 -5.1-11.3
Beclazone Easi-Breathe® 250 µg 2 doses optimiser	47.51	3.9	
Becloforte 1 dose APLUS	29.88	3.86	5.1 -3.1-13.3
Becloforte 2 doses APLUS	24.76	3.61	

a) Qvar 50 µg 2 doses aerosol APLUS versus Qvar 1 dose Easi-Breathe® 100 µg APLUS

* p<0.05, ** <0.01, ***<0.001, otherwise no significant difference.

However, as the results in Table 4.4 shows there is no significant difference in total emitted dose for the pMDI alone, Becloforte 1 dose and Becloforte 2

doses, Qvar 100 µg Easi-Breathe ® 1 dose and Qvar 100 µg Easi-Breathe® 2 doses and Qvar 50 µg 1 dose and Qvar 50 µg 2 doses. When 2 doses were required the patient leaflet instructions were followed (see appendix A). .

Furthermore, the effect of the number of actuations has been investigated on the delivered dose, using spacers. Four inhalers representing CFC and CFC-free and two spacers which are prone to static have been examined. There is no significant difference between 1 and 2 doses Table 4.4. Also, the data showed no significant difference between Qvar 50 µg 2 doses aerosol APLUS and Qvar 1 dose Easi-Breathe 100µg APLUS.

4.1.4 Effect of spacers on delivered dose

Tables 4.5 to 4.8 and Figures 4.4 to 4.7 show the summary of performance of the spacers with a different brand of beclomethasone.

All the investigated spacers decrease the delivered dose compared to an pMDI alone. There are also strong statistical differences, which are often $p < 0.001$ and many studies show the same effect. Barry and co-workers (1996) examined inhalation drug delivery from seven different spacers and found that they reduced the total amount of drug delivered from spacers.

Table 4.5 Average and ±STDEV of delivered dose percentage (% nominal dose) from different formulations of Qvar® with different spacers. Mean difference (95% confidence interval) for delivered dose of pMDI alone compared to delivered dose of pMDI+ spacers. (for Qvar alone data see Table 4.3)

Experiment (n=10)	Delivered dose (%)	STDEV	Mean difference
Qvar 50 µg 1 dose APLUS	47.64	4.18	-37.0*** -46.6- -27.4
Qvar 50 µg 2 doses APLUS	50.72	5.16	-35.5*** -45.1- -26.0
Qvar 50 µg 2 doses AMAX	76.19	7.81	-10.1* -19.6 - -.492
Qvar 100 µg 1 dose Optimiser®	51.04	6.64	-50.3*** -59.9 - -40.7
Qvar 100 µg 2 doses Optimiser®	64.12	18.92	-37.2*** -46.8 - -27.6
Qvar 100 µg 1 dose Easi-Breathe AMAX	93.98	11.89	-7.4 -16.9- 2.2
Qvar 100 µg 1 dose Easi-Breathe Optimizer	86.38	6.69	-15.0** -24.5- -5.4
Qvar 100 µg 1 dose Easi-Breathe APLUS	63.32	11.13	-23.1*** -32.6- -13.5

* p<0.05, ** <0.01, ***<0.001, otherwise no significant difference.

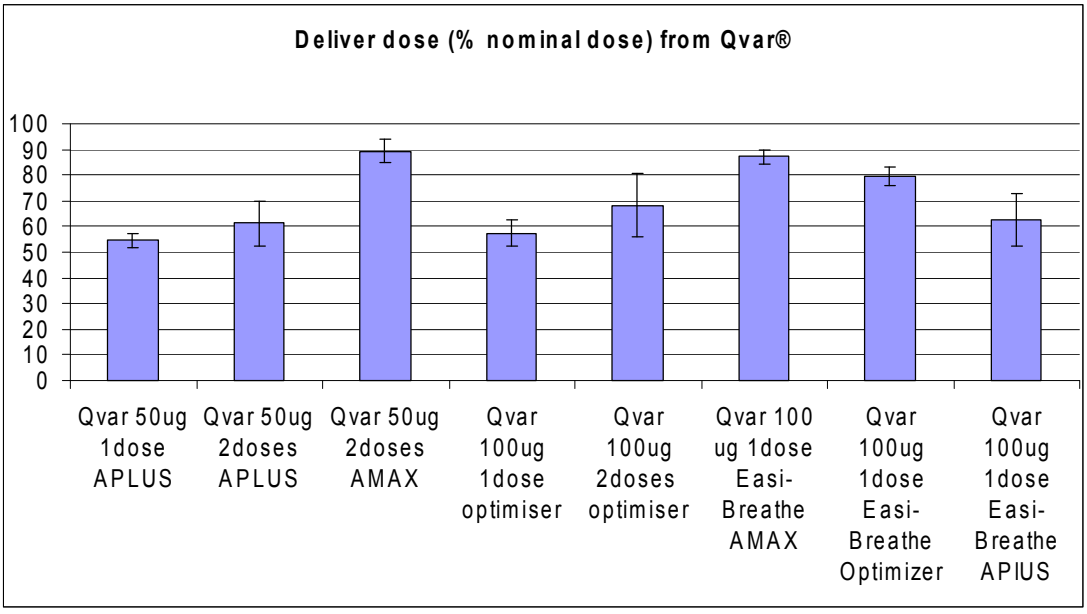

Figure 4.4 Delivered dose (% nominal dose) from Qvar® different formulations attached to different spacers.

Table 4.6 Average and ±STDEV of delivered dose percentage (% nominal dose) from different formulations of Clenil® with different spacers. Mean difference (95% confidence interval) for delivered dose of pMDI alone compared to delivered dose of pMDI+ spacers. (for Clenil® alone data see Table 4.3)

Experiment (n=10)	Delivered Dose (%)	STDEV	Mean difference
Clenil 100 µg 2 doses APLUS	24.01	10.07	-73.4*** 83.0- -63.8
Clenil 100 µg 2 doses AMAX	73.60	10.92	-23.8*** -33.4- -14.2
Clenil 250 µg 1 dose APLUS	31.99	3.51	-63.7*** -73.3- -54.1
Clenil 250 µg 1 dose AMAX	56.47	7.17	-39.3*** -48.8- -29.7

* p<0.05, ** <0.01, ***<0.001, otherwise no significant difference.

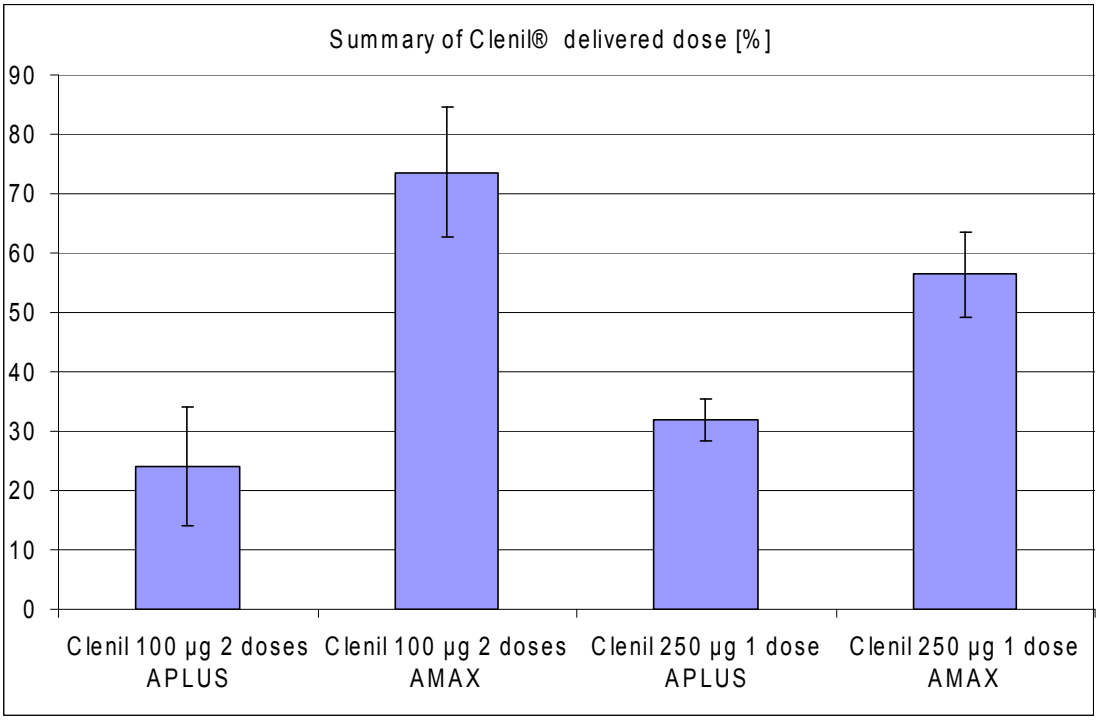

Figure 4.5 Total emitted dose (% nominal dose) from Clenil®, different concentration attached to different spacers.

Table 4.7 Average and ±STDEV of delivered dose percentage (% nominal dose) from different formulations of Beclazone® with different spacers. Mean difference (95% confidence interval) for delivered dose of pMDI alone compared to delivered dose of pMDI+ spacers. (for Beclazone® alone data see Table 4.3)

Experiment (n=10)	Delivered Dose (%)	STDEV	Mean difference
Beclazone100 µg 2 doses APLUS	44.31	9.22	-56.3*** -65.9- -46.7
Beclazone100 µg 2 doses AMAX	71.03	6.73	-29.6*** 39.15- -20.0
Beclazone Easi-Breathe® 250 µg 2 doses Optimizer	39.66	5.46	-53.9*** -63.5- -44.3

* p<0.05, ** <0.01, ***<0.001, otherwise no significant difference.

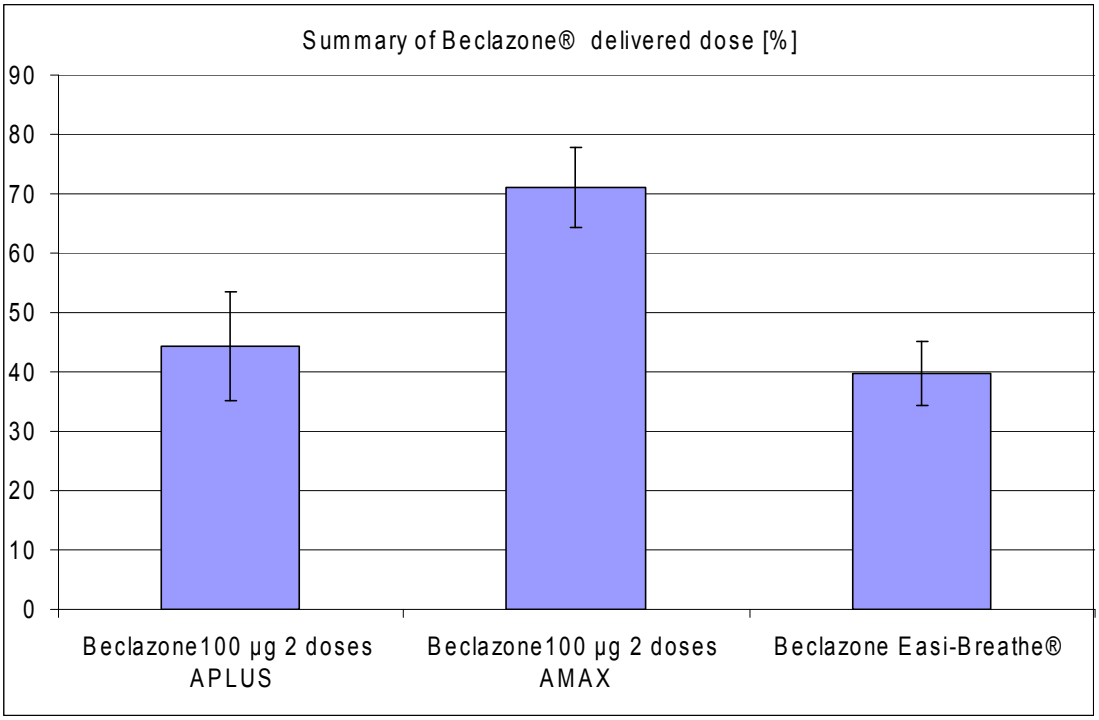

Figure 4.6 Total emitted dose (% nominal dose) from Beclazone®, different formulations attached to different spacers.

Table 4.8 Average and ±STDEV of delivered dose percentage (% nominal dose) from different formulations of Becotide® and Becloforte® with different spacers. Mean difference (95% confidence interval) for delivered dose of pMDI alone compared to delivered dose of pMDI+ spacers. (for Becotide® and Becloforte® alone data see Table 4.3)

Experiment (n=10)	Delivered Dose (%)	STDEV	Mean difference
Becotide100 µg 2 doses AMAX	40.95	7.78	-57.1*** -66.7- -47.6
Becotide100 µg 2 doses optimiser	59.36	6.84	-75.6*** -85.1- -66.0
Becloforte 1 dose APLUS	30.10	2.92	-70.9* -80.5-61.4
Becloforte 2 doses APLUS	23.01	4.89	-77.6*** -87.1- -68.0
Becloforte 1 dose AMAX	59.49	18.82	-41.5*** -51.1- -32.0

* p<0.05, ** <0.01, ***<0.001, otherwise no significant difference.

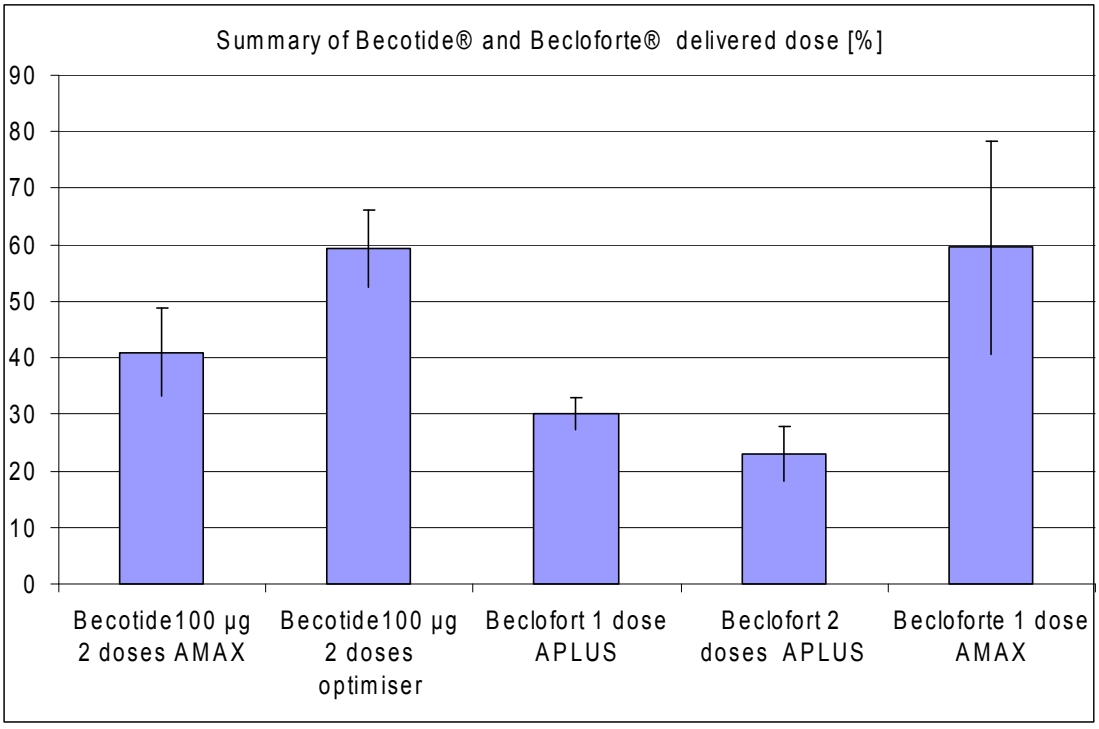

Figure 4.7 Total emitted dose (% nominal dose) from Becotide® and Becloforte® different formulations attached to different spacers.

Moreover, Nagel et al reported a reduction in the total delivered dose with fluticasone and salmeterol from 102.1 ug and 20 ug for pMDIs alone to 58.4 ug and 10.8 ug with APLUS respectively (Nagel et al., 2002). Hardy et al examined optimizer performance with three Easi-Breath ® Beclazone formulations. Using the impinger they demonstrated that the spacer removed 27% - 39% of the total dose. Also, their data showed a mean of 55% of the dose was deposited in the spacer, which was assayed using an imaging technique (gamma camera, Transmission images with technetium-99m) (Hardy et al., 1996).

4.1.5 Effect of type of VHCs and spacer

Electrostatic charge is created on discharging the aerosol, which can influence deposition in the spacer. Moreover, different spacers have different electrostatic properties. Non-electrostatic devices have been recommended for young children as these result in increased lung deposition (Devadason, 2006).

Table 4.9 Effect of type of spacer (95% confidence interval) on percentage of delivered dose (%emitted dose) for Becloforte® and Becotide®

Experiment (n=10)	Delivered dose (%)	STDEV	Mean difference
Becloforte 1 dose APLUS	29.88	3.86	-31.6***
Becloforte 1 dose AMAX	61.44	12.65	-39.8 - -23.3
Becotide100 µg 2 doses AMAX	68.05	3.77	26.4***
Becotide100 µg 2 doses optimiser	41.66	4.46	18.2- 34.6

* p<0.05, ** <0.01, ***<0.001, otherwise no significant difference.

Tables 4.9 to 4.12 and Figures 4.8 to 4.11 summarise the performance of the spacers with different formulations of beclomethasone. The data show statistically significant differences between spacer performances except in the

case of Qvar 100 µg Easi-Breathe ® with AMAX and Optimizer where the difference is not statistically significant. The AMAX spacers delivered the highest dose of all formulations, while the Optimizer performed better than APLUS.

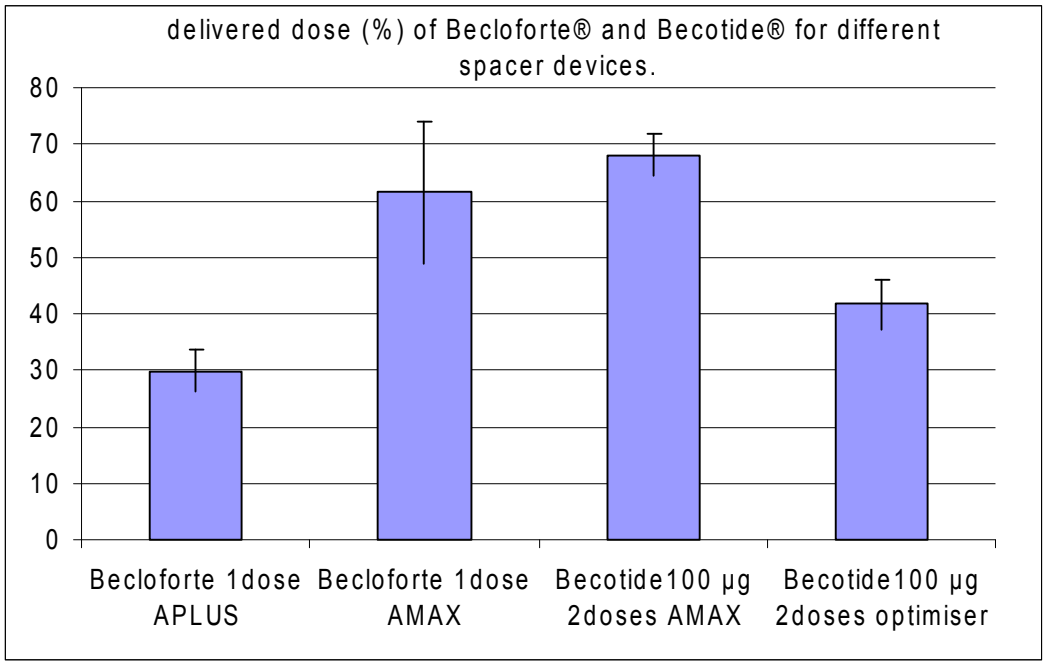

Figure 4.8 Percentage of delivered emitted dose of Becloforte® and Becotide® for different spacer devices.

Table 4.10 The effect of type of spacer (95% confidence interval) on percent of delivered dose (% emitted dose) for Qvar®.

Experiment (n=10)	Delivered dose (%)	STDEV	Mean difference
Qvar 50 µg 2 aerosol doses APLUS	61.17	8.50	-28.1*** -36.4- -19.9
Qvar 50 µg 2 doses aerosol AMAX	89.31	4.57	
Qvar 100 µg 1 dose Easi-Breathe AMAX	87.24	2.68	7.8 -0.4- -16.0[a]
			24.6*** 16.4-32.9[b]
Qvar 100 µg 1 dose Easi-Breathe Optimizer	79.44	3.54	-7.8 -16.0-0.4[c]
			16.8*** 8.6-25.1[d]
Qvar 100 µg 1 dose Easi-Breathe APLUS	62.62	10.20	-24.6*** -32.9- -16.4[e]
			-16.8*** -25.1- -8.6[e]

a AMAX vs. optimizer, b AMAX vs. APLUS, c optimizer vs. AMAX d optimizer vs. APLUS, e APLUS vs. AMAX, f APLUS vs. optimizer.

* p<0.05, ** <0.01, ***<0.001, otherwise no significant difference.

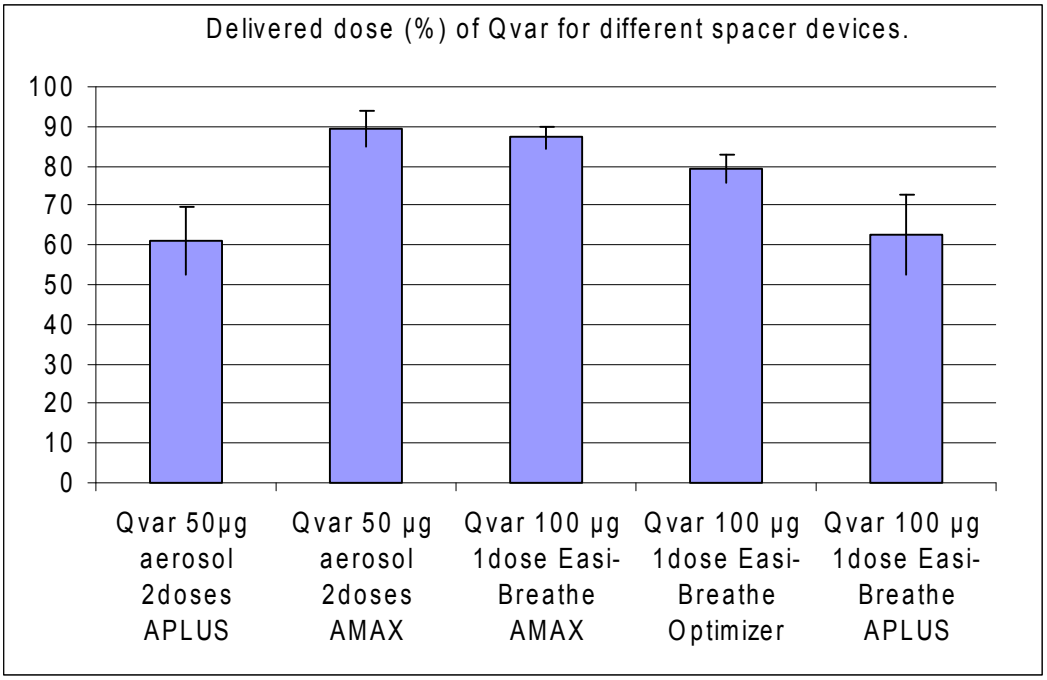

Figure 4.9 Percentage of delivered emitted dose of Qvar® for different spacer devices

Table 4.11 Effect of type of spacer (95% confidence interval) on percentage of delivered dose (%emitted dose) for Clenil®

Experiment (n=10)	Delivered Dose (%)	STDEV	Mean difference
Clenil 100 µg 2 doses APLUS	28.05	8.20	-49.5***
Clenil 100 µg 2 doses AMAX	77.55	2.29	-57.7 -41.3
Clenil 250 µg 1 dose APLUS	37.25	5.50	-29.1***
Clenil 250 µg 1 dose AMAX	66.41	3.57	-37.4- -20.9

* p<0.05, ** <0.01, ***<0.001, otherwise no significant difference.

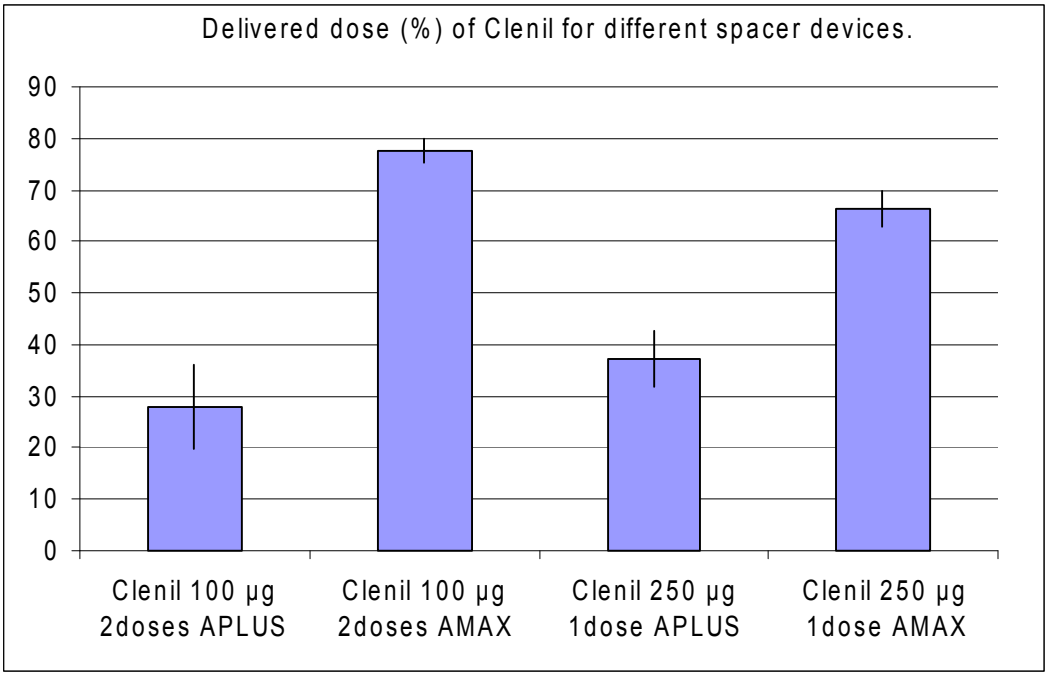

Figure 4.10 Percentage of delivered emitted dose of Clenil® for different spacer devices.

Table 4.12 Effect of type of spacer (95% confidence interval) on percentage of delivered dose (emitted dose) for Beclazone®.

Experiment (n=10)	Delivered Dose (%)	STDEV	Mean difference
Beclazone100 µg 2 doses APLUS	52.47	8.63	-15.6*** -23.8- -7.4
Beclazone100 µg 2 doses AMAX	68.05	3.57	

* p<0.05, ** <0.01, ***<0.001, otherwise no significant difference.

It is proposed that the half life of the aerosol available for inhalation is reduced by electrostatic activity resulting in a reduction in the delivered dose. Furthermore, the aerosol half life is 10s with the plastic spacers, while it is 30s if the static charge is abolished (Bisgaard et al., 2002). This agrees with the work of Terzano who reported that antistatic spacers deliver a significantly higher lung dose than ordinary spacers (Terzano, 2001). In addition, Anhoj and co-workers (1999) examined the effect of electrostatic charges *in-vivo* on

the lung dose of salbutamol in children. The plasma level of salbutamol was measured before and 5, 10, 15 and 20 min after inhalation of four single doses of 100 μg salbutamol. C_{max} and C_{av} 5—20 (min) were used as a reflection of lung deposition The results show that the dose of salbutamol had to be halved when an ordinary plastic spacer was used compared with the same spacer after antistatic priming.

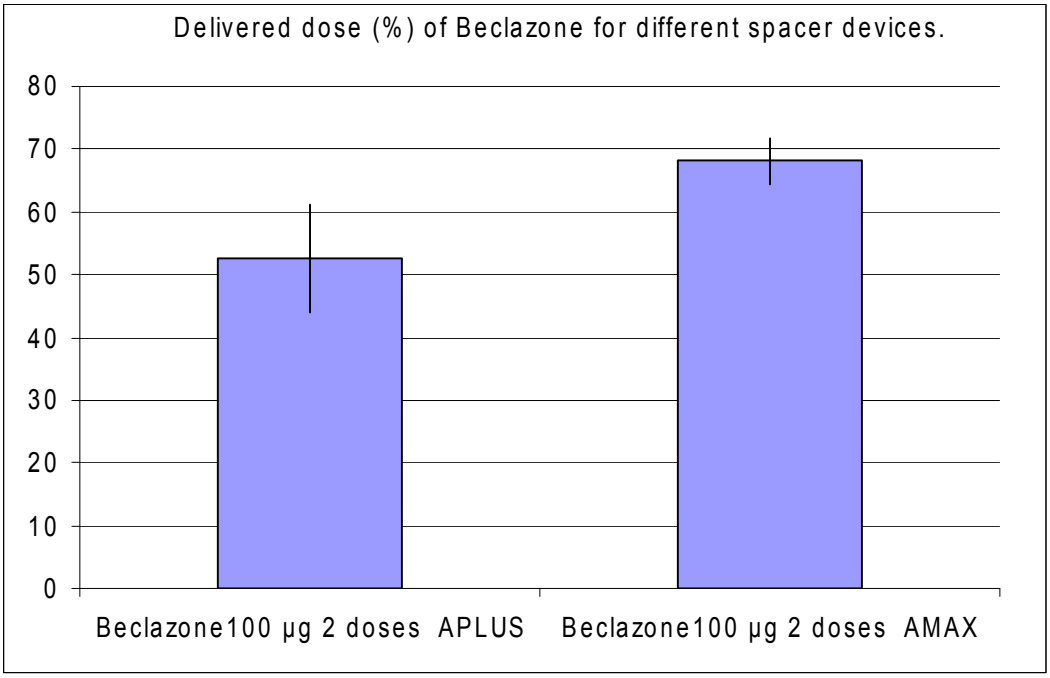

Figure 4.11 Percentage of delivered emitted dose of Beclazone® for different spacer devices.

Geller and co-workers (2006) tested the predicted lung delivery in infants of Flovent® CFC-free inhaler (fluticasone propionate) using the AMAX, Pari Vortex® (antistatic coating), and OptiChamber® Advantage (no antistatic treatment) as significantly AMAX delivered more Flovent® than the other two chambers. Geller and co-workers (2006) suggested that the results could be due to the lower chamber static and better valve design for AMAX. Hardy and co-workers (1996) measured the drug amount deposited in an optimizer

spacer with Qvar 100 and 50 ug. The result shows that the spacer deposition was 27% and 34% for 50 and 100 ug respectively. Iula and co-workers (1996) tested the performance of four different spacers coupled with Azmacort® (triamcinolone acetonide) and found up to a fivefold differences in the amount of drug delivered when using different spacers. Barry and co-workers (1996) demonstrated large variations in the lung dose delivered from different spacers and variations in the performance of spacers to deliver different drug.

4.1.6 Drug formulation

The reformulation of inhalers with HFA-based propellants has resulted in changes to the aerosol plume formulation characteristics.

Table 4.13 Effect of drug formulation and concentration on percentage of delivered dose (emitted dose) using AMAX

Formulation n=10)	Spacer (%)	Delivered dose (%)	STDEV
Qvar 50 ug 2 doses	10.69	89.31	4.33
Qvar 100 ug Easi-Breathe	12.76	87.24	2.55
Beclazone100 ug 2 doses	14.42	85.58	3.39
Clenil 100 ug 2 doses	22.45	77.55	2.17
Becotide100 ug 2 doses	31.95	68.05	3.58
Clenil 250 ug 1 dose	33.59	66.41	3.39
Becloforte ug 1dose	38.56	61.44	2.71

Drug formulation and drug concentration per actuation are important factors which influence drug delivery to the lung. Table 4.1 lists the excipients and alcohol content per actuation for each inhaler used in this study. The tested inhalers can be classified in three groups; first Qvar CFC-free containing alcohol and HFA; second, Clenil using Modulite® technology and it consists of

HFA, alcohol and glycerol. Modulite® technology uses the following variables to adjust the particle size: the non-volatile components of a solution formula (glycerol), the volume of the metered solution, the actuator orifice size and the vapour pressure of the propellants. The aim of this formulation is to maintain the same particle size of the CFC formulation (Bousquet, 2002). Third is a formulation which comprises CFC (Becloforte, Becotide and Beclazone).

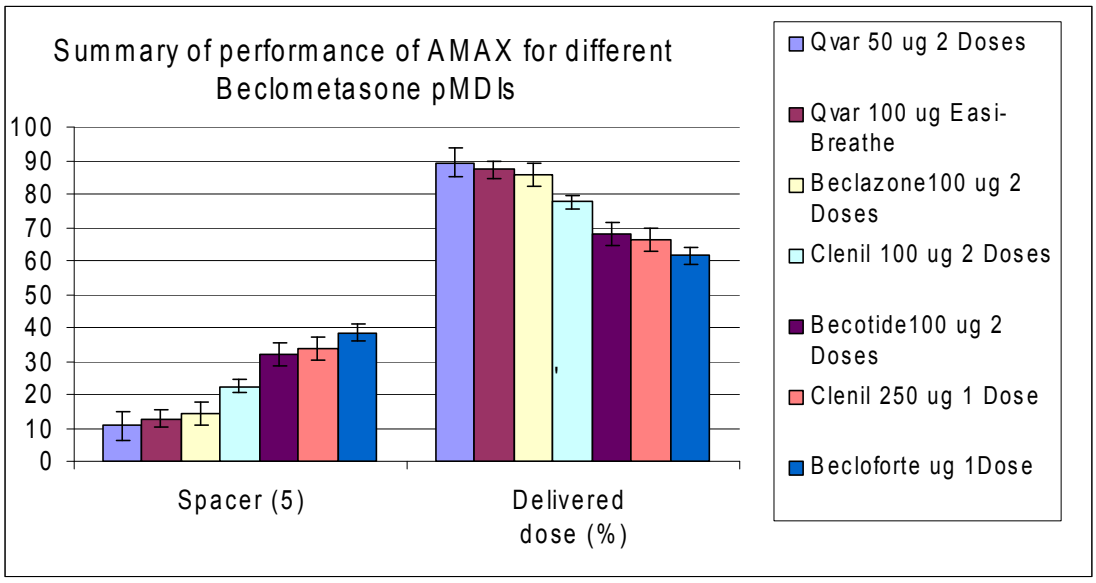

Figure 4.12 Effect of drug formulation and concentration on percent of delivered dose using AMAX.

Table 4.14 Effect of drug formulation and concentration on percentage of delivered dose (emitted dose) using Optimizer spacer.

Formulation	Spacer (%)	Collecting tube (%)	STDEV
Qvar 100 Easi-Breathe	20.56	79.44	3.54
Qvar 100 2 doses	31.72	68.29	12.34
Qvar 100 1 dose Aerosol	42.52	57.48	4.90
Beclazone Easi-Breathe® 250 ug 1 dose	49.38	50.62	3.44
Beclazone Easi-Breathe® 250 ug 2 doses	52.49	47.51	3.86
Becotide 100 ug 2 doses	58.34	41.66	7.13

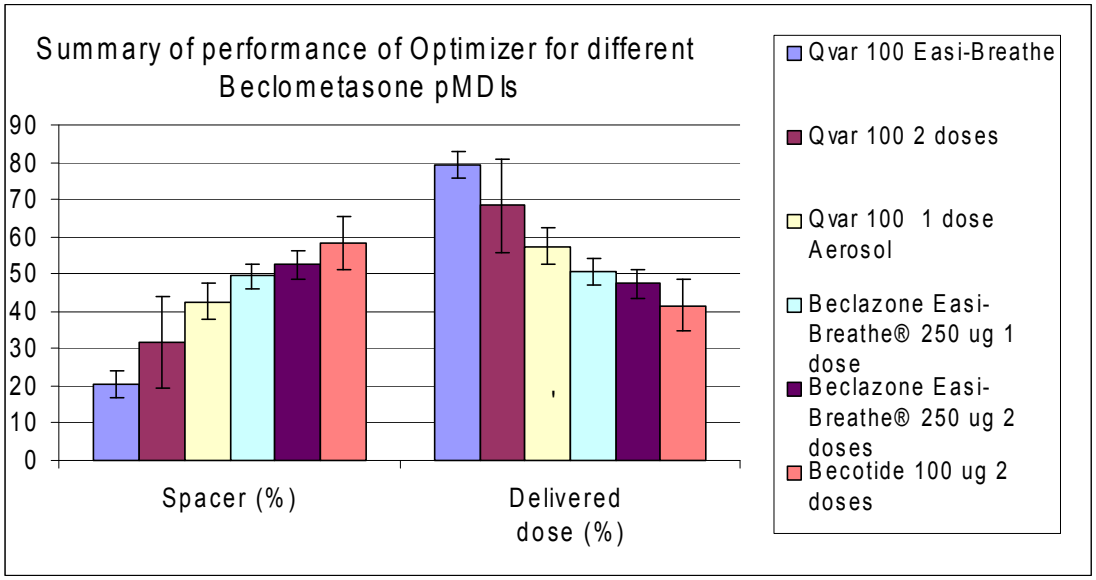

Figure 4.13 The effect of drug formulation and concentration on percentage of delivered dose using optimizer spacer

Table 4.15 The effect of drug formulation and concentration on percent of delivered (emitted dose) dose using APLUS

Formulation (n=10)	Spacer (%)	Delivered dose (%)	STDEV
Qvar 100 ug Easi-Breathe	37.38	62.62	10.20
Qvar 50 ug 2 Doses	38.84	61.17	8.50
Qvar 50 ug 1 Dose	45.48	54.52	2.87
Beclazone100 ug 2 Doses	47.53	52.47	8.63
Clenil 250 ug 1 Dose	62.75	37.25	5.50
Becloforte 1 Dose	70.12	29.88	3.86
Clenil 100 ug 2 Doses	71.96	28.05	8.20
Becloforte 2 Doses	75.24	24.76	3.61

Tables 4.13 to 4.18 and Figures 4.12 to 4.14 illustrated the effect of drug formulation. The results of AMAX show that the highest delivered dose was 89.31% with Qvar 50 ug and the lowest was 61.44% with Becloforte. On the other hand, the highest drug delivery with the optimizer spacer was 79.44%, with a Qvar 100 ug Easi-Breathe®, while the lowest was Becotide 100 ug with 41.66%. Also, the APLUS results show superiority of Qvar over other

formulations where Qvar Easi-Breathe® delivered 62.62%. These differences were statistically significant (see Tables 4.16 to 4.18).

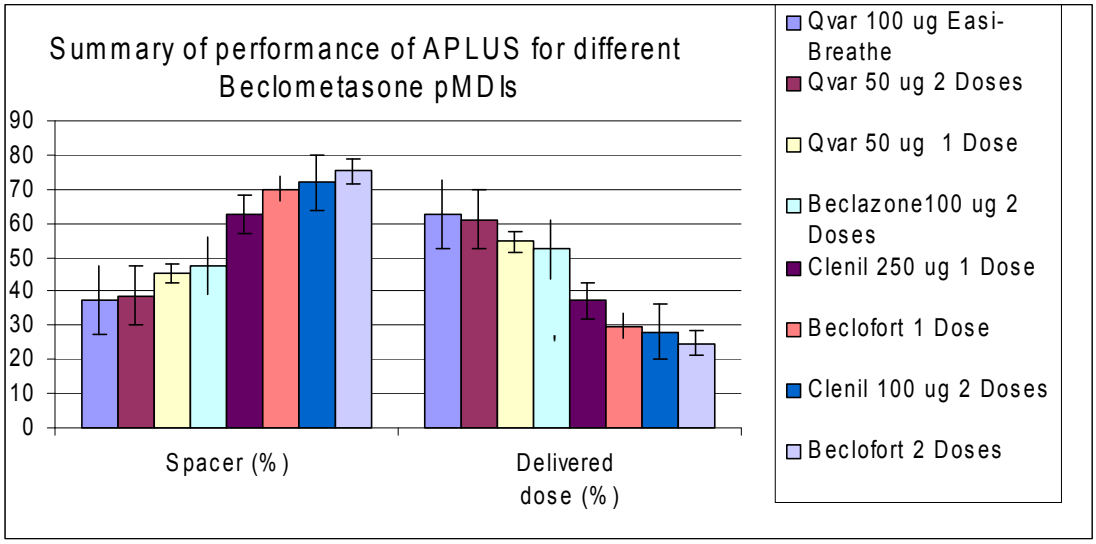

Figure 4.14 The effect of drug formulation and concentration on percentage of delivered dose (emitted dose) using APLUS.

Bisgaard and co-workers (2002) reported that the micronised drug particles are wet and surrounded by propellant which evaporates inside the VHC/ spacer. The superiority of Qvar can possibly be explained by its volatile content. Because it contains ethanol which easily evaporates and the amount of drug available for entrainment will therefore be more.

Table 4.16 Mean difference for total delivered dose (95% confidence interval) of performance (total delivered dose emitted dose)) of beclomethasone formulations with AMAX spacer.

Formulation	QE1D	Q52D	C21D	C1D	BT12D	BF1D	BZ12D
Beclazone100 2 Doses BZ12D)	-19.2** -36.0- -2.4	-21.3** -38.1- -4.4	1.6 -15.2- 18.5	-9.5 -26.3- 7.3	0.0 -16.8- 16.8	6.6 -10.2- 23.5	++++
Becloforte 1Dose BF1D)	-25.8*** -42.6- -9.0	-27.9*** -44.7- -11.0	-5.0 -21.8- 11.9	-16.1 -32.9- 0.7	-6.6 -23.5- 10.2	++++	-6.6 -23.5- 10.2
Becotide100 2 Doses BT12D)	-19.2** -36.0- -2.4	-21.3** -38.1- -4.4	1.6 -15.2- 18.5	-9.5 -26.3- 7.3	++++	6.6 -10.2- 23.5	0.0 -16.8-16.8
Clenil 100 2 Doses C1D)	-9.7 -26.5- 7.1	-11.8 -28.6- 5.1	11.1 -5.7- 28.0	++++	9.5 -7.3- 26.3	16.1 -0.7- 32.9	9.5 -7.3- 26.3
Clenil 250mcg 1 Dose C21D)	-20.8** -37.7- -4.0	-22.9*** -39.7- -6.1	++++	-11.1 -28.0- 5.7	-1.6 -18.5- 15.2	5.0 -11.9- 21.8	-1.6 -18.5- 15.2
Qvar 50 2 Doses Q52D)	2.1 -14.8-18.9	++++	22.9*** 6.1- 39.7	11.8 -5.1- 28.6	21.3** 4.4- 38.1	27.9*** 11.0- 44.7	21.3** 4.4- 38.1
Qvar Easi-Breathe QE1D)	++++	-2.1 -18.9- 14.8	20.8** 4.0- 37.7	9.7 -7.1- 26.5	19.2** 2.4- 36.0	25.8*** 9.0- 42.6	19.2** 2.4- 36.0

* p<0.05, ** <0.01, ***<0.001, otherwise no significant difference.

Table 4.17 Mean difference of performance of beclomethasone formulations (total delivered dose emitted dose)) with optimizer spacer

Formulation	QE1D	Q12D	Q11D	BT12D	BZE22D	BZE21D
Beclazone Easi-Breathe® 250 mcg 1 dose BZE21D)	-28.8*** -45.7- -12.0	-17.7* -34.5- -0.8	-6.9 -23.7- 10.0	9.0 -7.9- 25.8	3.1 -13.7- 19.9	++++
Beclazone Easi-Breathe® 250 mcg 2 doses BZE22D)	-31.9*** -48.8- -15.1	-20.8** -37.6- -3.9	-10.0 -26.8- 6.9	5.9 -11.0- 22.7	++++	-3.1 -19.9- 13.7
Becotide 100 mcg 2 doses BT12D)	-37.8*** -54.6- -20.9	-26.6*** -43.5- -9.8	-15.8 -32.7- 1.0	++++	-5.9 -22.7- 11.0	-9.0 -25.8- 7.9
Qvar 100 1 dose Q11D)	-22.0*** -38.8- -5.1	-10.8 -27.6- 6.0	++++	15.8 -1.0- 32.7	10.0 -6.9- 26.8	6.9 -10.0- 23.7
Qvar 100 2 doses Q12D)	-11.2 -28.0- 5.7	++++	10.8 -6.0- 27.6	26.6*** 9.8- 43.5	20.8** 3.9- 37.6	17.7* 0.8- 34.5
Qvar Easi-Breathe QE1D)	++++	11.2 -5.7- 28.0	22.0*** 5.1- 38.8	37.8*** 20.9- 54.6	31.9*** 15.1- 48.8	28.8*** 12.0- 45.7

* $p<0.05$, ** <0.01, ***<0.001, otherwise no significant difference.

Table 4.18 Mean difference of performance of beclomethasone formulations (total delivered dose emitted dose)) with APLUS.

Formulation	QE11D	Q52D	Q51D	C21D	C12D	BF2D	BF1D	BZ12D
Beclazone100 2 Doses BZ12D)	-10.1 -27.0- 6.7	-8.7 -25.5- 8.1	-2.1 -18.9- 14.8	15.2 -1.6- 32.1	24.4*** 7.6- 41.3	27.7*** 10.9- 44.6	22.6*** 5.7- 39.4	++++
Becloforte 1 Dose BF1D)	-32.7*** -49.6- -15.9	-31.3*** -48.1- -14.4	-24.6*** -41.5- -7.8	-7.4 -24.2- 9.5	1.8 -15.0- 18.7	5.1 -11.7- 22.0	++++	-22.6*** -39.4- -5.7
Becloforte 2 Doses BF2D)	-37.9*** -54.7- -21.0	-36.4*** -53.2- -19.6	-29.8*** -46.6- -12.9	-12.5 -29.3- 4.3	-3.3 -20.1- 13.6	++++	-5.1 -22.0- 11.	-27.7*** -44.6- -10.9
Clenil 100 2 Doses C12D)	-34.6*** -51.4- -17.7	-33.1*** -50.0- -16.3	-26.5*** -43.3- -9.6	-9.2 -26.0- 7.6	++++	3.3 -13.6- 20.1	-1.8 -18.7- 15.	-24.4*** -41.3- -7.6
Clenil 250 mcg 1 Dose C21D)	-25.4*** -42.2- -8.5	-23.9*** -40.8- -7.1	-17.3* -34.1- -0.4	++++	9.2 -7.6- 26.0	12.5 -4.3- 29.3	7.4 -9.5- 24.2	-15.2 -32.1- 1.6
Qvar 50 1 Dose Q51D)	-8.1 -24.9- 8.7	-6.6 -23.5- 10.2	++++	17.3* 0.4- 34.1	26.5*** 9.6- 43.3	29.8*** 12.9- 46.6	24.6*** 7.8- 41.5	2.1 -14.8- 18.9
Qvar 50 2 Doses Q52D)	-1.5 -18.3- 15.4	++++	6.6 -10.2- 23.5	23.9*** 7.1- 40.8	33.1*** 16.3- 50.0	36.4*** 19.6- 53.2	31.3*** 14.4- 48.1	8.7 -8.1- 25.5
Qvar Easi-Breathe QE11D)	++++	1.5 -15.4- 18.3	8.1 -8.7- 24.9	25.4*** 8.5- 42.2	34.6*** 17.7- 51.4	37.9*** 21.0- 54.7	32.7*** 15.9- 49.6	10.1 -6.7- 27.0

* p<0.05, ** <0.01, ***<0.001 otherwise no significant difference

CONCLUSION

The phasing-out of CFC pMDIs has promoted introducing new formulations. As a result it is necessary to show that the new formulations do not alter the quality, efficacy and safety profiles of the inhalers. This process begins by demonstrating that the alternative propellant does not adversely affect dosage uniformity or pulmonary deposition.

Establishing the performance of new products in these terms is essential to demonstrate that new CFC-free pMDIs meet the regulatory requirements.

It is possible that the new formulations may affect the characteristic of the aerosol plume development by several factors which are formulation-dependent, including propellant type, vapour pressure, type of excipients and actuator nozzle size. As a consequence, the interaction of the aerosol particles with spacers could result in a change of the drug deposition within spacers. There are also many other factors which may affect drug deposition within spacers, which are spacer dependent, including electrostatic charge, volume and the shape of the spacer, incorporated valves and the materials used to build the spacer.

This study results show that experiments with one spacer or drug cannot be extrapolated directly to other spacers or drugs, therefore it is necessary to test specific drugs and device combinations. The use of a universal spacer common to all inhalers, even if the pMDI adapter fits, will lead to arbitrary dosing. Therefore, it is suggested that the regulatory authorities consider an pMDI to be used only with the spacer tested. In addition, the spacer leaflet should include the brand names of acceptable pMDIs with which it has been tested.

CHAPTER 5

PERFORMANCE OF SEVERAL BRAND NAMES OF BECLOMETHASONE pMDIs WITH DIFFERENT SPACERS

5 PERFORMANCE OF SEVERAL BRAND NAMES OF BECLOMETHASONE pMDIs WITH DIFFERENT SPACERS

OBJECTIVES

The objective of this section of the research program is to compare the *in-vitro* aerosol deposition characteristics from different beclomethasone pMDIs with three common spacers. Also, to evaluate how these spacers affect the dose of beclomethasone delivered to the lungs and the throat deposition. A secondary objective is to examine the hypothesis of whether the result of a specific spacer with a given drug/ brand name can be extrapolated to other pMDIs or brand names for the same drug.

INTRODUCTION

As indicated earlier considerable formulation changes were needed when switching to HFA 134a. The new formulations were in solution rather than a micronised suspension as in the CFC pMDI. Also, design changes were needed within the HFAs, pMDI canister, valve and actuator. In conjunction with these changes, it was essential to evaluate the particle size distribution and performance of the new product.

As suggested at the beginning of this thesis, particle size is obviously a crucial factor in inhaled drugs, affecting both the lung dose and delivery location and therefore clinical efficacy. It has been proposed that the primary factor of drug deposition in the lung is its aerodynamic size. It characterises the particle's inertial behaviour and its rate of sedimentation because particles range about some average size, they are generally expressed as a mass median aerodynamic diameter (MMAD) and sizes of 1 to 5 μm are usually considered

as suitable for pulmonary delivery. Particles at the high end of this range would deposit centrally in the larger conducting airways whereas fine particles would reach the pulmonary periphery. In many formulations, the fraction of the cloud in this size range is usually expressed as the fine particle dose (FPD), i.e. the fraction of the label claim < 5 μm (Ganderton et al., 2002).

The phasing-out of CFC pMDIs led to the adoption of different strategies: first it is possible to generate clouds close to or having similar particle size and drug mass CFC formulations which lead to a seamless transition; second to use this opportunity to refine and produce a clinically useful product with defined particle size targeting specific lung areas (Bousquet, 2002).

There are many brands of generic beclomethasone dipropionate pMDIs available in the UK. However, there has been controversy as to the in equivalence (Barnes et al., 1996).

Although, the local side effects of inhaled corticosteroids are considered minor problems. However, while not generally serious, they are clinically important, because they may hamper compliance with therapy. They include dysphonia, oropharyngeal candidiasis, thirst, cough, tongue hypertrophy and peri-oral dermatitis. On the other hand, the cold Freon effect, in which the cold high-velocity aerosol impacts on the back of the throat, can cause patients to stop inhaling prematurely. The use of a spacer may reduce these effects or eliminate them. However, the spacer may cause peri-oral dermatitis, especially when a mask is used (Roland et al., 2004).

METHODOLOGY

5.1.1 Instrumentation

5.1.1.1 Equipment and inhalation device

The equipment used for the aerodynamic study is described in section 2.1 2.6.

The analytical HPLC method is detailed in section 2.6.

5.1.1.2 Instrumentation set-up

The initial work was to prepare the ACI. All its parts and stages were washed with acetone and dried. Furthermore, to ensure efficient capture of the particles collection plates were sprayed with silicone (USP, 2005) and allowed to air dry. The ACI stages were then assembled as described in the manufacture manual which incorporates an after filter below the final stage to capture any fine particles which otherwise would escape from the apparatus.

Figure 5.1 shows the positioning of the ACI, critical flow controller and vacuum pump. The pMDIs/spacer was connected to the mouthpiece adapter. Then they were attached to the end of the induction port. The flow control valve was adjusted to achieve a steady flow through the system at the required rate 28.3L/min (±5%) which was measured by an electronic digital flow meter Model (DFM). According to the pharmacopeial method 4L of air were drawn through the inhaler for each determination and the absolute pressure ratio P3/P2 < 0.5, was confirmed.

The pMDI/spacer was then prepared according to the patient leaflet instructions (see appendix A). The mean content of drug per actuation was tested at different points between the first and final actuation.

Figure 5.1 The setting of Sampling Apparatus, Critical Flow Controller, and Vacuum Pump.

Groups of a five doses were selected randomly using random schedules for studying the aerodynamic size over the entire set of inhalers. Each dose of five was separately discharged into the apparatus by opening the valve if the device was breath-actuated, otherwise it was done by pressing down on the inhaler to release its contents. The time 8.4 sec (±5%) was calculated according to equation 4-1. Determinations were made for each pMDI and for each pMDI attached to each of the three different spacers (n=5). The spacers were Optimizer®, Aerochamber® MAX® (AMAX) and Aerochamber® PLUS® (APLUS). Table 5.1 summarises the experiments. The apparatus was then dismantled carefully to avoid any loss of material. The active ingredient was washed from the inner walls and the collection plate of each of the stages of the apparatus into an appropriate volume of washing solution (acetonitrile : water 70:30 v/v) Table 5.2.

Table 5.1 List of experiments.

Experiment (n=5)
Beclazone alone
Beclazone AMAX
Beclazone APLUS
Beclazone Optimizer
Becloforte alone
Becloforte AMAX
Becloforte APLUS
Becloforte Optimizer
Clenil alone
Clenil AMAX
Clenil APLUS
Clenil Optimizer
Qvar alone
Qvar AMAX
Qvar APLUS
Qvar Optimizer

The drug under test was extracted from the filter into the washing solution. In order to ensure complete extraction the filter was sonicated for three minutes in the washing solution. Also, the washing solution from the filter was further filtered through a 0.45 µm filter in order to remove any unwanted particles which might block the HPLC system.

Table 5.2 The washing volume of 70% acetonitrile for ACI stages.

Stage	Volume of washing (ml)	
	pMDI	pMDI with spacers
Spacer	-	50
Induction port	50	25
0	10	10
1	25	25
2	25	25
3	25	25
4	25	25
5	10	10
6	10	10
7	10	10
Filter	10	10

The amount of drug was determined by HPLC using the previously validated method described in section 2.6. Spacer and each ACI stage content is reported separately.

5.1.2 Fine particle analysis

All the aerodynamic calculations were conducted using the Copley software (CITDAS version 2).

5.1.3 Statistical analysis

The one-way ANOVA with Bonferroni effect test was used to compare the aerodynamic particle size characterization of pMDIs and pMDIs with spacer using SPSS V15.0 (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

Four inhalers representing CFC and CFC-free, and two spacers which are prone to static and an anti-static spacer have been examined. Beclazone® and Becloforte® are CFC pMDIs whereas Clenil® Modulite® and Qvar® are CFC-free. In addition, the Optimizer® and APLUS prone to static and AMAX anti-static spacer for more detail, see section 4.1. Table 3.1 shows stage cut size (μm) for ACI for 28.3L/min.

For comparative purposes, the throat deposition percentage was calculated as the amount of drug deposited in the induction port expressed as a percentage of the product label claim. Also, the FPD was expressed as a percentage of the nominal dose. Tables 5.3 to 5.10 and Figures 5.2 to 5.9 summarised the aerodynamic results while the Tables 5.11 to 5.18 and Figures 5.10 to 5.17 are comparisons of beclomethasone pMDIs aerodynamic characterisations.

Table 5.3 Amounts (μg) of beclomethasone using five doses, deposited on each stage of the ACI, from Clenil® 250 μg alone and with different spacers

	ALONE		AMAX		OPTIMIZER		APLUS	
Stage (n=5)	AVERAGE	STDEV	AVERAGE	STDEV	AVERAGE	STDEV	AVERAGE	STDEV
Spacer			75.83	17.65	122.82	3.85	161.10	22.30
Throat [ug]	168.80	23.85	24.79	3.24	9.66	2.48	8.02	2.31
Stage 0 [ug]	7.28	1.86	2.92	0.31	8.01	7.10	0.60	0.47
Stage 1 [ug]	3.82	0.30	10.47	1.29	3.93	1.48	5.76	2.20
Stage 2 [ug]	6.14	1.07	19.08	1.16	7.87	1.64	12.74	3.48
Stage 3 [ug]	18.70	3.86	46.67	5.00	19.82	3.93	38.52	9.83
Stage 4 [ug]	19.09	1.61	35.18	2.49	19.14	0.93	36.75	6.28
Stage 5 [ug]	16.26	2.01	21.18	2.33	16.07	1.70	20.99	2.96
Stage 6 [ug]	4.38	0.33	3.71	0.65	2.98	0.57	3.55	0.25
Stage 7 [ug]	2.18	0.43	1.40	0.55	1.08	0.67	0.68	0.75
Filter [ug]	1.58	0.30	0.95	0.70	0.96	0.09	1.47	0.28
Ex-mouth dose [ug]	248.22	20.71	242.21	26.24	212.36	6.19	290.19	38.75
Delivered Dose [ug]	248.22	20.71	166.37	8.69	89.54	4.97	129.10	20.58
Fine Particle Dose [ug]	63.88	5.60	115.72	6.56	62.56	2.84	106.76	18.02
Fine Particle Fraction [%]	25.93	3.53	69.55	1.10	69.94	3.12	82.61	1.75
MMAD [um]	3.01	0.26	3.52	0.11	3.25	0.18	3.16	0.21
GSD	1.86	0.10	1.47	0.04	1.60	0.03	1.49	0.05

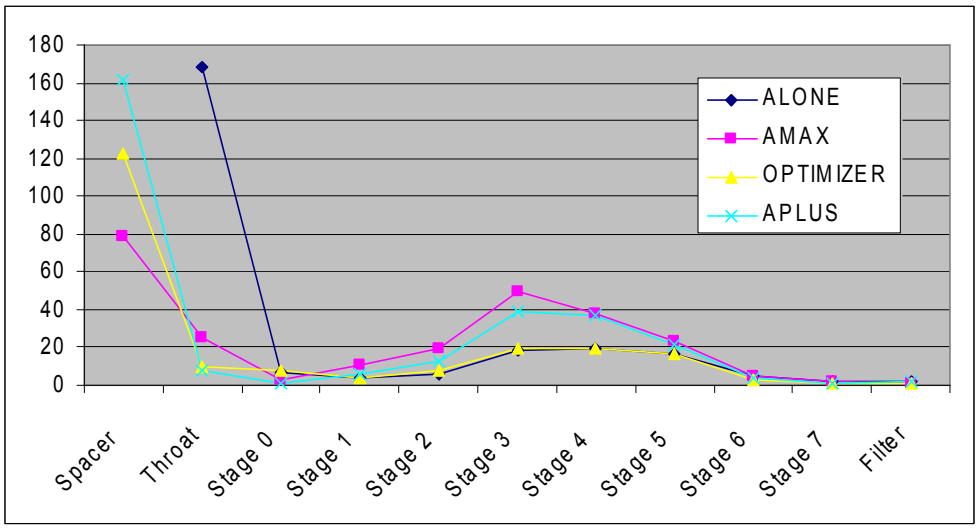

Figure 5.2 Amount μg) of beclomethasone deposited on each stage of the ACI from Clenil® alone and with different spacers.

Table 5.4 Cumulative mass percentage under size for Clenil® alone and with different spacers.

Stage	ALONE	AMAX	OPTIMIZER	APLUS
Stage 0 [%]	100.00	100.00	100.00	100.00
Stage 1 [%]	90.83	98.02	89.97	99.51
Stage 2 [%]	86.02	90.86	85.04	94.75
Stage 3 [%]	78.29	78.07	75.19	84.22
Stage 4 [%]	54.75	45.42	50.37	52.41
Stage 5 [%]	30.72	20.18	26.41	22.05
Stage 6 [%]	10.25	4.76	6.28	4.71
Stage 7 [%]	4.73	1.81	2.55	1.78
Filter [%]	1.99	0.76	1.20	1.22

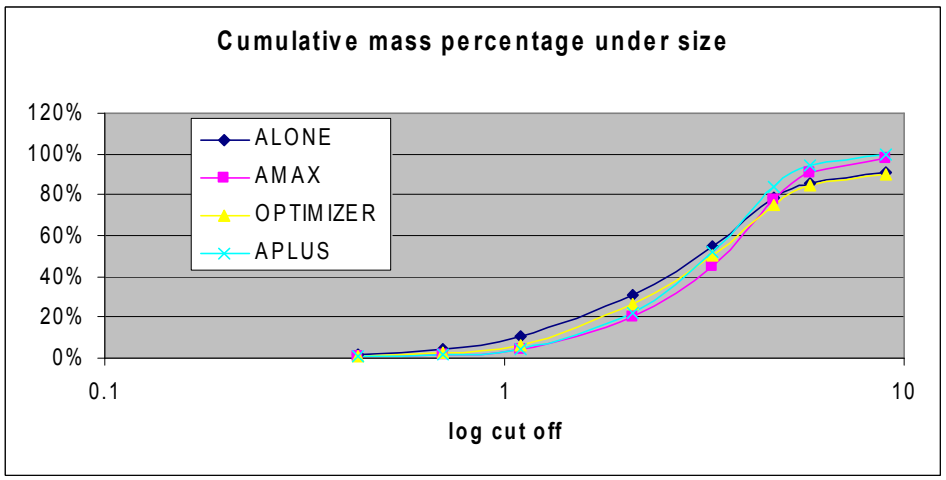

Figure 5.3 Cumulative mass percentage under size for beclomethasone deposited on each stage of the ACI from Clenil® alone and with different spacers.

Table 5.5 Amounts µg) of beclomethasone using five doses, deposited on each stage of the ACI, from Becloforte® 250 µg alone and with different spacers.

	ALONE		AMAX		OPTIMIZER		APLUS	
Stage n=5)	AVERAGE	STDEV	AVERAGE	STDEV	AVERAGE	STDEV	AVERAGE	STDEV
Spacer			127.10	17.02	181.24	29.39	185.94	20.62
Throat [ug]	204.20	27.87	34.80	5.13	15.80	2.32	19.47	1.85
Stage 0 [ug]	16.09	2.37	7.39	1.81	9.68	1.28	5.67	1.42
Stage 1 [ug]	22.22	4.28	24.25	3.74	20.40	1.82	17.71	2.19
Stage 2 [ug]	20.28	1.47	26.96	13.42	22.23	2.20	20.99	0.38
Stage 3 [ug]	31.28	1.94	40.80	16.00	29.13	1.15	38.55	8.05
Stage 4 [ug]	9.13	1.85	16.35	10.45	10.07	1.99	13.46	3.16
Stage 5 [ug]	0.64	0.76	1.78	0.87	1.26	0.53	1.96	0.53
Stage 6 [ug]	0.00	0.00	0.07	0.13	0.17	0.28	0.06	0.09
Stage 7 [ug]	0.00	0.00	0.03	0.05	0.00	0.00	0.00	0.00
Filter [ug]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ex-mouth dose [ug]	303.84	27.68	279.53	58.22	289.98	36.13	303.81	8.47
Delivered Dose [ug]	303.84	27.68	152.43	46.90	108.74	8.75	117.87	12.42
Fine Particle Dose [ug]	46.77	4.17	67.37	30.26	47.10	4.42	60.82	11.27
Fine Particle Fraction [%]	15.39	1.44	44.20	9.79	43.31	0.75	51.60	4.17
MMAD [um]	5.15	0.16	4.69	0.43	4.97	0.06	4.50	0.19
GSD	1.41	0.21	1.48	0.03	1.57	0.07	1.49	0.03

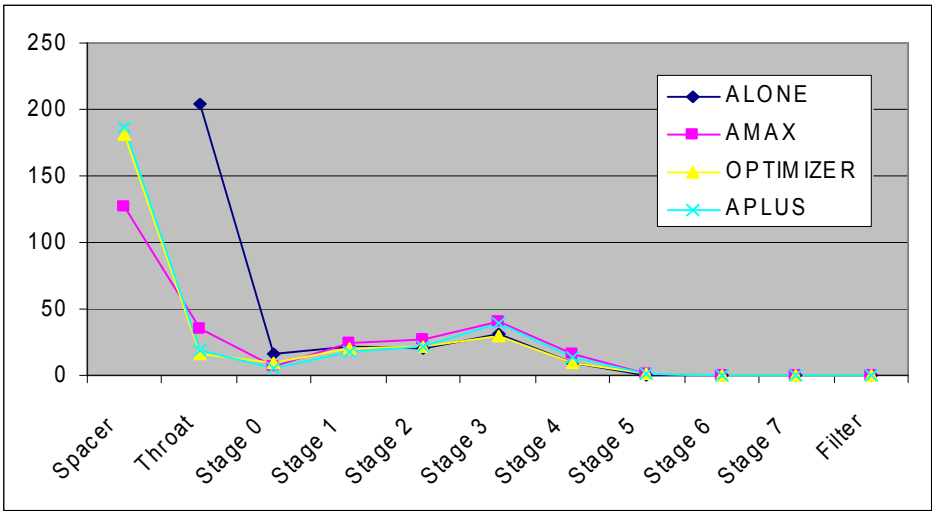

Figure 5.4 Amount (μg) of beclomethasone deposited on each stage of the ACI from Becloforte® alone and with different spacers.

Table 5.6 Cumulative mass percentage under size for Becloforte® alone and with different spacers.

Stage	ALONE	AMAX	OPTIMIZER	APLUS
Stage 0 [%]	100.00	100.00	100.00	100.00
Stage 1 [%]	83.86	93.72	89.58	94.24
Stage 2 [%]	61.55	73.10	67.64	76.24
Stage 3 [%]	41.21	50.18	43.72	54.91
Stage 4 [%]	9.82	15.50	12.39	15.74
Stage 5 [%]	0.65	1.60	1.55	2.06
Stage 6 [%]	0.01	0.09	0.19	0.07
Stage 7 [%]	0.01	0.03	0.01	0.01
Filter [%]	0.00	0.00	0.01	0.00

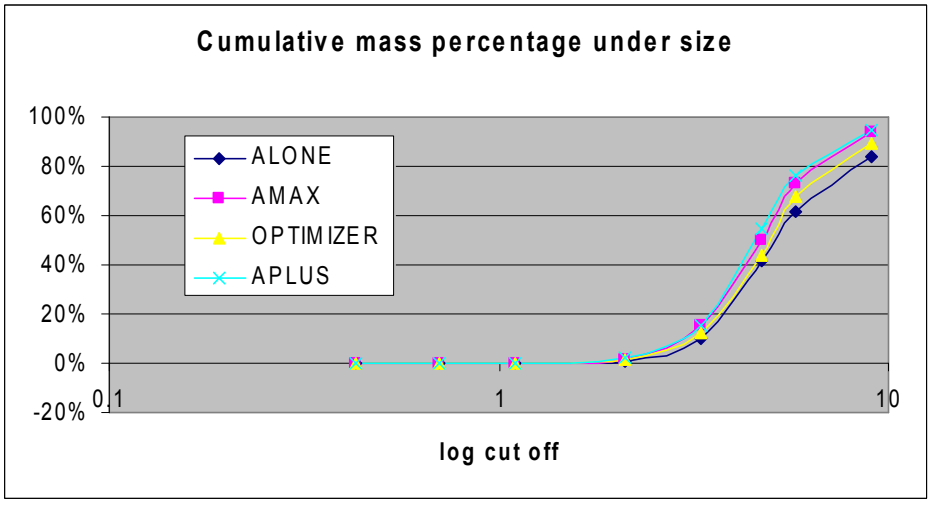

Figure 5.5 Cumulative mass percentage under size for beclomethasone deposited on each stage of the ACI from Becloforte® alone and with different spacers.

Table 5.7 Amounts (μg) of beclomethasone using five doses, deposited on each stage of the ACI, from Beclazone® 250 μg alone and with different spacers.

	ALONE		AMAX		OPTIMIZER		APLUS	
Stage (n=5)	AVERAGE	STDEV	AVERAGE	STDEV	AVERAGE	STDEV	AVERAGE	STDEV
Spacer			73.48	11.37	119.54	11.08	150.05	38.84
Throat [ug]	162.41	21.36	32.00	5.71	8.70	1.34	10.85	2.04
Stage 0 [ug]	19.55	18.40	9.61	0.11	22.78	2.64	10.62	0.89
Stage 1 [ug]	38.98	33.16	27.58	3.23	27.35	2.02	22.22	2.69
Stage 2 [ug]	18.05	10.64	31.99	1.01	23.80	2.87	25.35	1.62
Stage 3 [ug]	26.38	8.97	36.88	2.25	30.39	1.69	36.05	7.37
Stage 4 [ug]	9.65	1.19	8.57	0.46	8.01	0.84	10.24	0.92
Stage 5 [ug]	1.95	1.05	0.01	0.00	0.22	0.36	0.19	0.32
Stage 6 [ug]	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00
Stage 7 [ug]	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00
Filter [ug]	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00
Ex-mouth dose [ug]	277.00	25.03	220.15	11.58	240.82	18.94	265.60	45.26
Delivered Dose [ug]	277.00	25.03	146.67	6.58	121.28	8.24	115.55	6.52
Fine Particle Dose [ug]	42.71	12.46	54.61	2.28	45.14	3.10	53.93	8.92
[%]	15.42	5.61	37.23	1.50	37.22	0.75	46.68	5.22
MMAD [um]	5.88	1.47	5.08	0.09	5.49	0.00	4.94	0.19
GSD	1.58	0.12	1.47	0.01	1.42	0.02	1.56	0.03

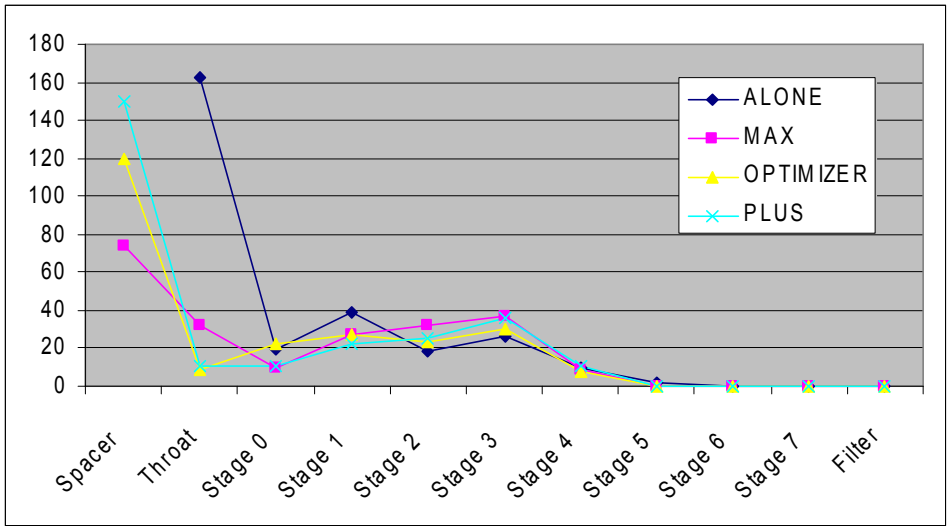

Figure 5.6 Amount (μg) of beclomethasone deposited on each stage of the ACI from Beclazone® alone and with different spacers.

Table 5.8 Cumulative mass percentage under size for Beclazone® alone and with different spacers.

Stage	ALONE	AMAX	OPTIMIZER	APLUS
Stage 0 [%]	100.0	100.0	100.0	100.0
Stage 1 [%]	82.94	91.62	79.76	89.86
Stage 2 [%]	48.92	67.57	55.47	68.63
Stage 3 [%]	33.17	39.67	34.32	44.42
Stage 4 [%]	10.14	7.50	7.32	9.99
Stage 5 [%]	1.72	0.02	0.21	0.20
Stage 6 [%]	0.02	0.02	0.02	0.02
Stage 7 [%]	0.01	0.01	0.01	0.01
Filter [%]	0.01	0.01	0.01	0.01

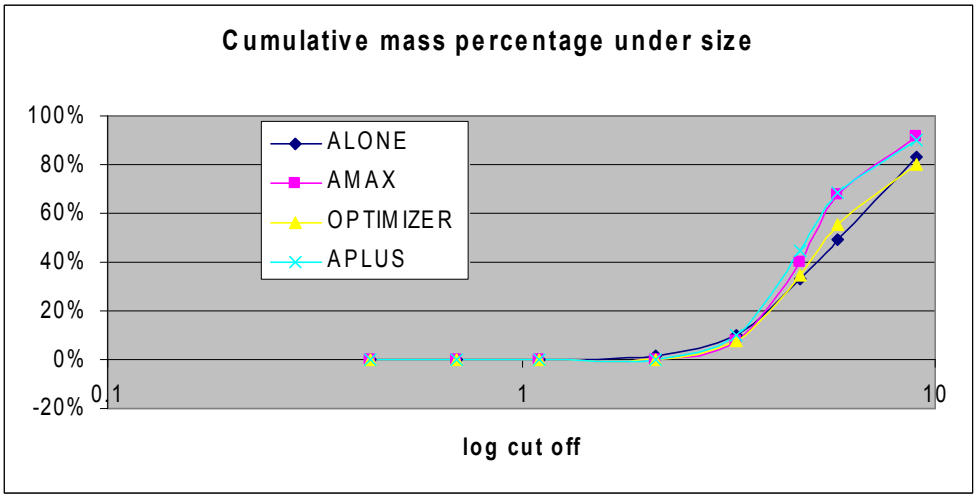

Figure 5.7 Cumulative mass percentage under size for beclomethasone deposited on each stage of the ACI from Beclazone® alone and with different spacers.

Table 5.9 Amounts (μg) of beclomethasone using five doses, deposited on each stage of the ACI, from Qvar® 100 μg alone and with different spacers.

	ALONE		AMAX		OPTIMIZER		APLUS	
Stage (n=5)	AVERAGE	STDEV	AVERAGE	STDEV	AVERAGE	STDEV	AVERAGE	STDEV
Spacer			14.07	3.52	28.15	4.27	30.65	5.99
Throat [ug]	28.67	5.84	2.72	0.52	3.61	0.09	0.33	0.28
Stage 0 [ug]	0.66	0.26	2.85	4.66	0.23	0.20	0.64	0.92
Stage 1 [ug]	0.16	0.19	0.13	0.22	0.09	0.08	0.05	0.09
Stage 2 [ug]	0.30	0.12	1.17	1.29	0.33	0.28	0.07	0.12
Stage 3 [ug]	1.07	0.33	2.78	0.83	0.63	0.40	0.26	0.22
Stage 4 [ug]	7.48	1.66	10.46	2.81	4.55	1.87	2.65	0.14
Stage 5 [ug]	29.17	3.29	27.66	0.50	24.38	2.70	20.97	3.92
Stage 6 [ug]	13.49	2.33	14.39	2.22	15.18	3.70	12.21	0.55
Stage 7 [ug]	5.26	0.12	4.97	1.37	5.90	1.09	5.30	2.05
Filter	3.39	0.72	3.54	0.16	3.78	0.90	6.07	1.19
Ex-mouth dose [ug]	89.63	3.76	84.74	7.49	86.83	0.65	79.20	3.09
Delivered Dose [ug]	89.63	3.76	70.67	5.05	58.68	4.75	48.55	5.32
Fine Particle Dose [ug]	59.60	6.31	64.30	5.19	53.97	4.75	47.26	4.80
Fine Particle Fraction [%]	66.49	6.52	90.98	5.22	91.97	0.95	97.33	1.36
MMAD [um]	1.31	0.04	1.43	0.07	1.17	0.10	1.11	0.06
GSD	1.62	0.06	1.90	0.28	1.60	0.04	1.55	0.06

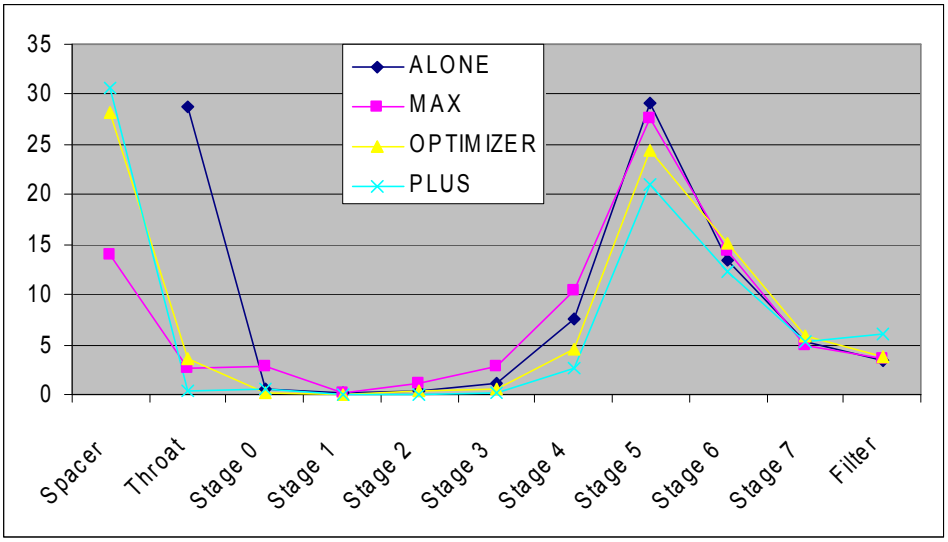

Figure 5.8 Amount (μg) of beclomethasone deposited on each stage of the ACI from Qvar® alone and with different spacers.

Table 5.10 Cumulative mass percentage under size for Qvar® alone and with different spacers.

Stage	ALONE	AMAX	OPTIMIZER	APLUS
Stage 0 [%]	100.0	100.0	100.0	100.0
Stage 1 [%]	98.92	95.81	99.58	98.67
Stage 2 [%]	98.66	95.62	99.41	98.56
Stage 3 [%]	98.16	93.90	98.81	98.41
Stage 4 [%]	96.41	89.81	97.67	97.87
Stage 5 [%]	84.14	74.42	89.41	92.38
Stage 6 [%]	36.30	33.71	45.15	48.89
Stage 7 [%]	14.18	12.53	17.58	23.57
Filter [%]	5.55	5.21	6.87	12.59

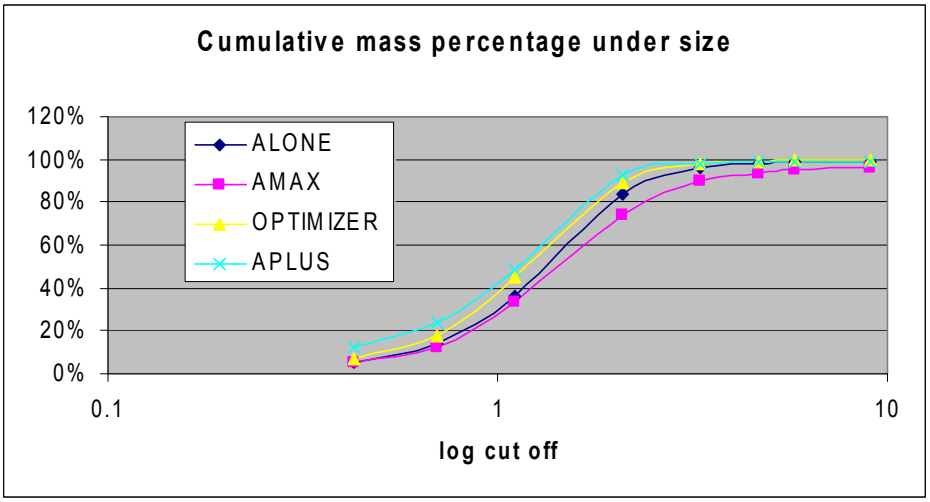

Figure 5.9 Cumulative mass percentage under size for beclomethasone deposited on each stage of the ACI from Qvar® alone and with different spacers.

Table 5.11 A comparison of the percentage of beclomethasone pMDIs alone deposited on each stage of the ACI.

	CLENIL		Becloforte		Becalzone		Qvar	
Stage (n=5)	AVERAGE	STDEV	AVERAGE	STDEV	AVERAGE	STDEV	AVERAGE	STDEV
Throat [%]	67.52	9.54	81.68	11.15	64.96	8.55	28.67	5.84
Stage 0 [%]	2.91	0.74	6.44	0.95	7.82	7.36	0.66	0.26
Stage 1 [%]	1.53	0.12	8.89	1.71	15.59	13.27	0.16	0.19
Stage 2 [%]	2.45	0.43	8.11	0.59	7.22	4.26	0.30	0.12
Stage 3 [%]	7.48	1.54	12.51	0.78	10.55	3.59	1.07	0.33
Stage 4 [%]	7.63	0.64	3.65	0.74	3.86	0.48	7.48	1.66
Stage 5 [%]	6.50	0.80	0.26	0.30	0.78	0.42	29.17	3.29
Stage 6 [%]	1.75	0.13	0.00	0.00	0.00	0.00	13.49	2.33
Stage 7 [%]	0.87	0.17	0.00	0.00	0.00	0.00	5.26	0.12
Filter [%]	0.63	0.12	0.00	0.00	0.00	0.00	3.39	0.72
Nominal Dose [ug]	250		250		250		100	
Ex-mouth dose [%]	99.29	8.28	121.54	11.07	110.80	10.01	89.63	3.76
Delivered Dose [%]	99.29	8.28	121.54	11.07	110.80	10.01	89.63	3.76
Fine Particle Dose [%]	25.55	2.24	18.71	1.67	17.08	4.98	59.60	6.31
Fine Particle Fraction [%]	25.74	3.53	15.39	1.44	15.42	5.61	66.49	6.52
MMAD [um]	3.01	0.26	5.15	0.16	5.88	1.47	1.31	0.04
GSD	1.86	0.10	1.41	0.21	1.58	0.12	1.62	0.06

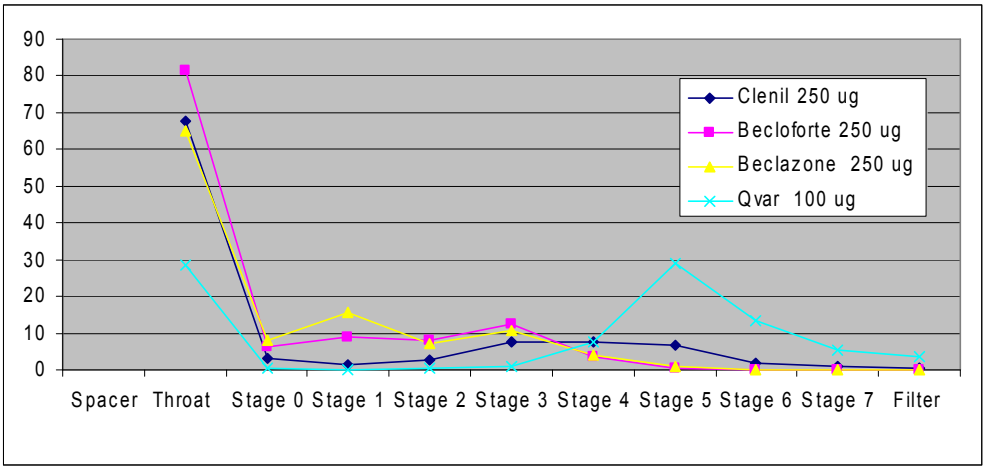

Figure 5.10 A comparison of the percentage of beclomethasone deposited on each stage of the ACI from the different pMDIs.

Table 5.12 A comparison of cumulative mass percentages under size of beclomethasone pMDIs alone deposited on each stage of the ACI.

Stage	Clenil	Becloforte	Beclazone	Qvar
Stage 0 [%]	100.00	100.00	100.00	100.00
Stage 1 [%]	90.83	83.86	82.94	98.92
Stage 2 [%]	86.02	61.55	48.92	98.66
Stage 3 [%]	78.29	41.21	33.17	98.16
Stage 4 [%]	54.75	9.82	10.14	96.41
Stage 5 [%]	30.72	0.65	1.72	84.14
Stage 6 [%]	10.25	0.01	0.02	36.30
Stage 7 [%]	4.73	0.01	0.01	14.18
Filter [%]	1.99	0.00	0.01	5.55

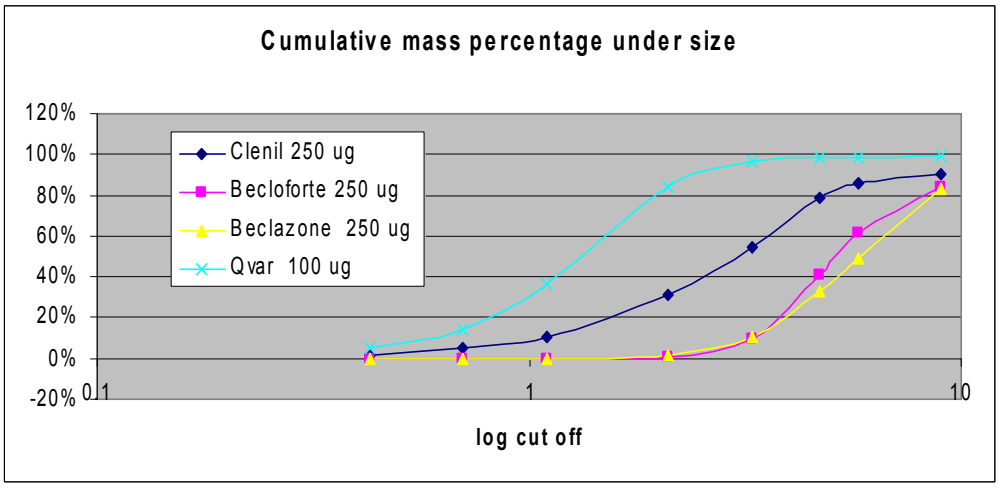

Figure 5.11 A comparison of cumulative mass percentages under size for beclomethasone pMDIs alone deposited on each stage of the ACI.

Table 5.13 A comparison of the percentage nominal dose) of beclomethasone MDIs with AMAX deposited on each stage of the ACI.

	Clenil		Becloforte		Beclazone		Qvar	
Stage	AVERAGE	STDEV	AVERAGE	STDEV	AVERAGE	STDEV	AVERAGE	STDEV
AMAX	31.56	6.70	50.84	6.81	29.39	4.55	14.07	3.52
Throat [%]	10.10	1.20	13.92	2.05	12.80	2.28	2.72	0.52
Stage 0 [%]	1.20	0.13	2.96	0.72	3.84	0.04	2.85	4.66
Stage 1 [%]	4.35	0.58	9.70	1.50	11.03	1.29	0.13	0.22
Stage 2 [%]	7.77	0.50	10.78	5.37	12.80	0.40	1.17	1.29
Stage 3 [%]	19.84	3.14	16.32	6.40	14.75	0.90	2.78	0.83
Stage 4 [%]	15.34	2.96	6.54	4.18	3.43	0.19	10.46	2.81
Stage 5 [%]	9.37	2.15	0.71	0.35	0.00	0.00	27.66	0.50
Stage 6 [%]	1.80	0.74	0.03	0.05	0.00	0.00	14.39	2.22
Stage 7 [%]	0.64	0.26	0.01	0.02	0.00	0.00	4.97	1.37
Filter [%]	0.46	0.30	0.00	0.00	0.00	0.00	3.54	0.16
Nominal Dose [ug]	250.00	0.00	250.00	0.00	250.00	0.00	100.00	0.00
Ex-mouth dose [%]	102.42	15.37	111.81	23.29	88.06	4.63	84.74	7.49
Delivered Dose [%]	70.87	10.12	60.97	18.76	58.67	2.63	70.67	5.05
Fine Particle Dose [%]	50.13	8.88	26.95	12.10	21.84	0.91	64.30	5.19
Fine Particle Fraction [%]	69.55	2.35	44.20	9.79	37.23	1.50	90.98	5.22
MMAD [um]	3.48	0.13	4.69	0.43	5.08	0.09	1.43	0.07
GSD	1.48	0.03	1.48	0.03	1.47	0.01	1.90	0.28

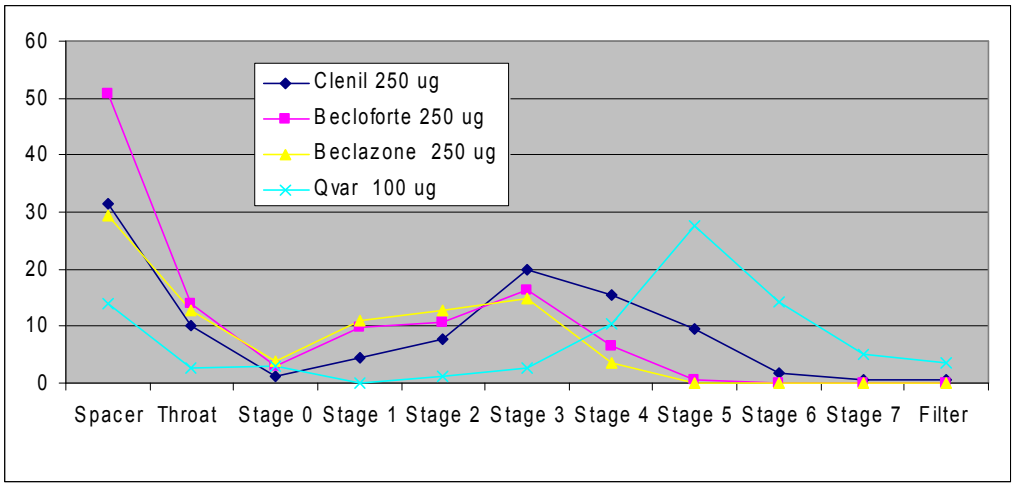

Figure 5.12 A comparison of the percentage of beclomethasone deposited on each stage of the ACI from the different pMDIs with AMAX.

Table 5.14 A comparison of cumulative mass percentages under size of beclomethasone pMDIs with AMAX deposited on each stage of the ACI.

Stage	Clenil	Becloforte	Beclazone	Qvar
Stage 0 [%]	100.00	100.00	100.00	100.00
Stage 1 [%]	98.02	93.72	91.62	95.81
Stage 2 [%]	90.86	73.10	67.57	95.62
Stage 3 [%]	78.07	50.18	39.67	93.90
Stage 4 [%]	45.42	15.50	7.50	89.81
Stage 5 [%]	20.18	1.60	0.02	74.42
Stage 6 [%]	4.76	0.09	0.02	33.71
Stage 7 [%]	1.81	0.03	0.01	12.53
Filter [%]	0.76	0.00	0.01	5.21

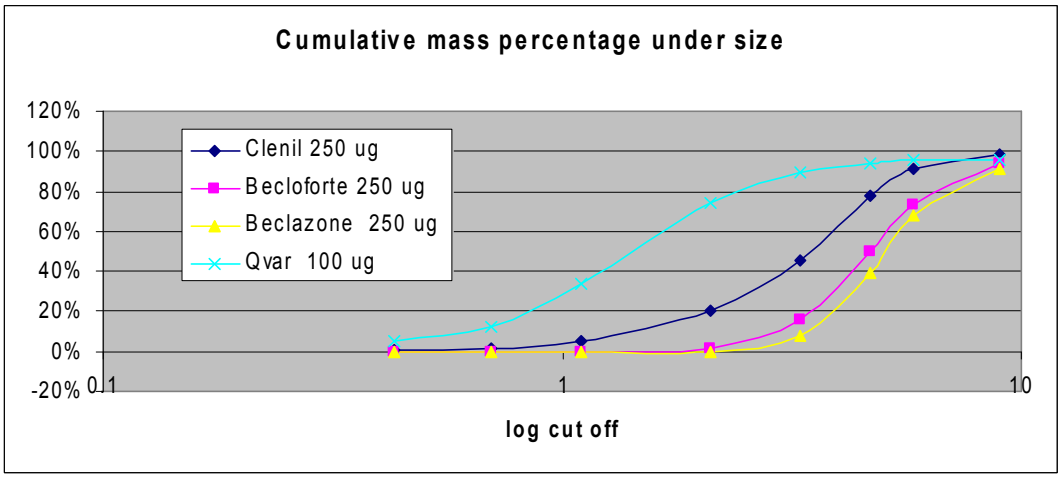

Figure 5.13 A comparison of cumulative mass percentages under size for beclomethasone pMDIs with AMAX deposited on each stage of the ACI.

Table 5.15 A comparison of the percentage nominal dose) of beclomethasone MDIs with Optimizer deposited on each stage of the ACI

	Clenil		Becloforte		Beclazone		Qvar	
Stage	AVERAGE	STDEV	AVERAGE	STDEV	AVERAGE	STDEV	AVERAGE	STDEV
Optimizer	49.13	1.54	72.50	11.76	47.81	4.43	28.15	4.27
Throat [%]	3.87	0.99	6.32	0.93	3.48	0.54	3.61	0.09
Stage 0 [%]	3.21	2.84	3.87	0.51	9.11	1.06	0.23	0.20
Stage 1 [%]	1.57	0.59	8.16	0.73	10.94	0.81	0.09	0.08
Stage 2 [%]	3.15	0.66	8.89	0.88	9.52	1.15	0.33	0.28
Stage 3 [%]	7.93	1.57	11.65	0.46	12.16	0.67	0.63	0.40
Stage 4 [%]	7.65	0.37	4.03	0.80	3.20	0.34	4.55	1.87
Stage 5 [%]	6.43	0.68	0.51	0.21	0.09	0.15	24.38	2.70
Stage 6 [%]	1.19	0.23	0.07	0.11	0.00	0.00	15.18	3.70
Stage 7 [%]	0.43	0.27	0.00	0.00	0.00	0.00	5.90	1.09
Filter [%]	0.38	0.03	0.00	0.00	0.00	0.00	3.78	0.90
Nominal Dose [ug]	250.00	0.00	250.00	0.00	250.00	0.00	100.00	0.00
Ex-mouth dose [%]	84.95	2.48	115.99	14.45	96.33	7.58	86.83	0.65
Delivered Dose [%]	35.82	1.99	43.50	3.50	48.51	3.30	58.68	4.75
Fine Particle Dose [%]	25.03	1.14	18.84	1.77	18.06	1.24	53.97	4.75
Fine Particle Fraction [%]	69.94	3.12	43.31	0.75	37.22	0.75	91.97	0.95
MMAD [um]	3.25	0.18	4.97	0.06	5.49	0.00	1.17	0.10
GSD	1.60	0.03	1.57	0.07	1.42	0.02	1.60	0.04

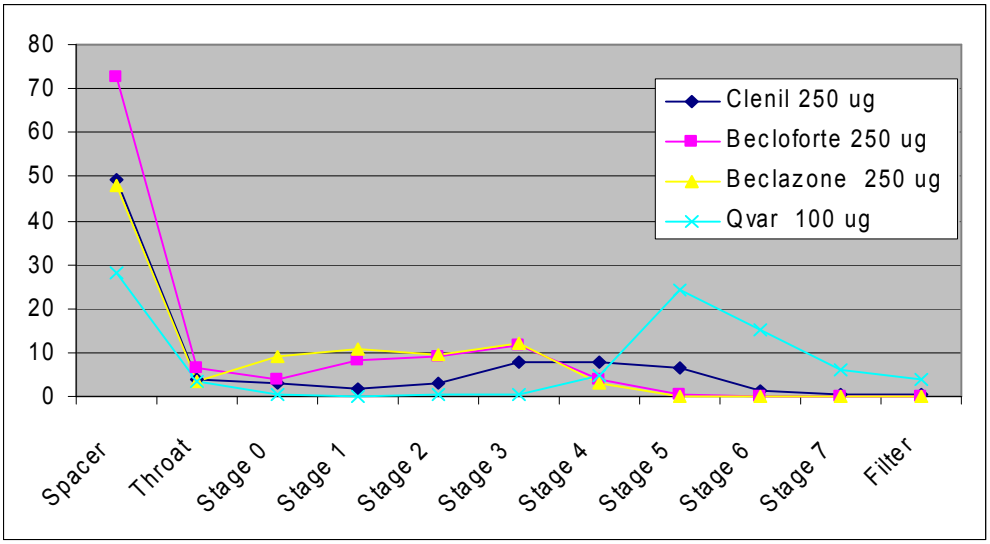

Figure 5.14 A comparison of the percentage of beclomethasone deposited on each stage of the ACI from the pMDIs with Optimizer.

Table 5.16 A comparison of cumulative mass percentages under size of beclomethasone pMDIs with Optimizer deposited on each stage of the ACI.

Stage	Clenil	Becloforte	Beclazone	Qvar
Stage 0 [%]	100.00	100.00	100.00	100.00
Stage 1 [%]	89.97	89.58	79.76	99.58
Stage 2 [%]	85.04	67.64	55.47	99.41
Stage 3 [%]	75.19	43.72	34.32	98.81
Stage 4 [%]	50.37	12.39	7.32	97.67
Stage 5 [%]	26.41	1.55	0.21	89.41
Stage 6 [%]	6.28	0.19	0.02	45.15
Stage 7 [%]	2.55	0.01	0.01	17.58
Filter [%]	1.20	0.01	0.01	6.87

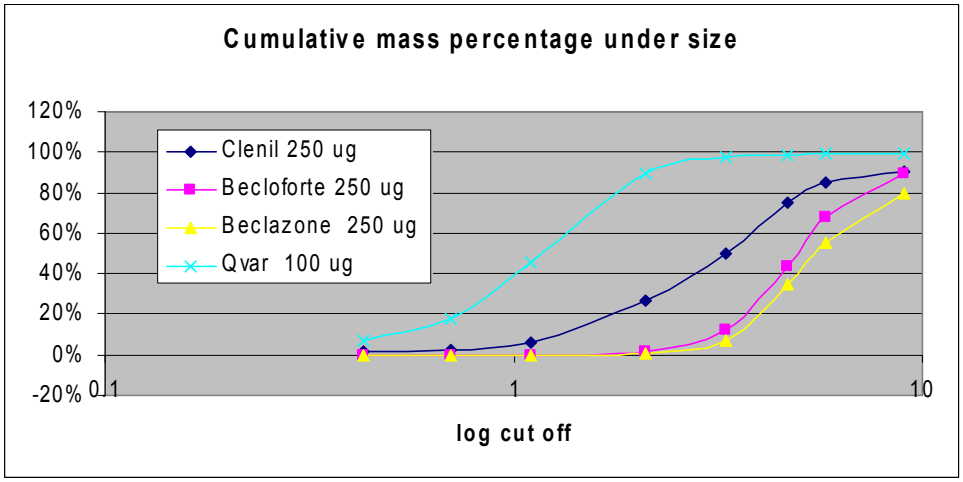

Figure 5.15 A comparison of cumulative mass percentages under size for beclomethasone from pMDIs with Optimizer deposited on each stage of the ACI.

Table 5.17 A comparison of the percentage nominal dose) of beclomethasone MDIs with APLUS deposited on each stage of the ACI.

	Clenil		Becloforte		Beclazone		Qvar	
Stage	AVERAGE	STDEV	AVERAGE	STDEV	AVERAGE	STDEV	AVERAGE	STDEV
APLUS	64.44	8.92	74.38	8.25	60.02	15.54	30.65	5.99
Throat [%]	3.21	0.92	7.79	0.74	4.34	0.82	0.33	0.28
Stage 0 [%]	0.24	0.19	2.27	0.57	4.25	0.36	0.64	0.92
Stage 1 [%]	2.30	0.88	7.08	0.88	8.89	1.07	0.05	0.09
Stage 2 [%]	5.10	1.39	8.40	0.15	10.14	0.65	0.07	0.12
Stage 3 [%]	15.41	3.93	15.42	3.22	14.42	2.95	0.26	0.22
Stage 4 [%]	14.70	2.51	5.38	1.27	4.10	0.37	2.65	0.14
Stage 5 [%]	8.40	1.18	0.79	0.21	0.08	0.13	20.97	3.92
Stage 6 [%]	1.42	0.10	0.02	0.04	0.00	0.00	12.21	0.55
Stage 7 [%]	0.27	0.30	0.00	0.00	0.00	0.00	5.30	2.05
Filter [%]	0.59	0.11	0.00	0.00	0.00	0.00	6.07	1.19
Nominal Dose [ug]	250.00	0.06	250.00	0.00	250.00	0.00	100.00	0.00
Ex-mouth dose [%]	116.07	15.50	121.52	3.39	106.24	18.11	79.20	3.09
Delivered Dose [%]	51.63	8.23	47.15	4.97	46.22	2.61	48.55	5.32
Fine Particle Dose [%]	42.70	7.21	24.33	4.51	21.57	3.57	47.26	4.80
Fine Particle Fraction [%]	82.71	1.75	51.60	4.17	46.68	5.22	97.33	1.36
MMAD [um]	3.18	0.21	4.50	0.19	4.94	0.19	1.11	0.06
GSD	1.48	0.05	1.49	0.03	1.56	0.03	1.55	0.06

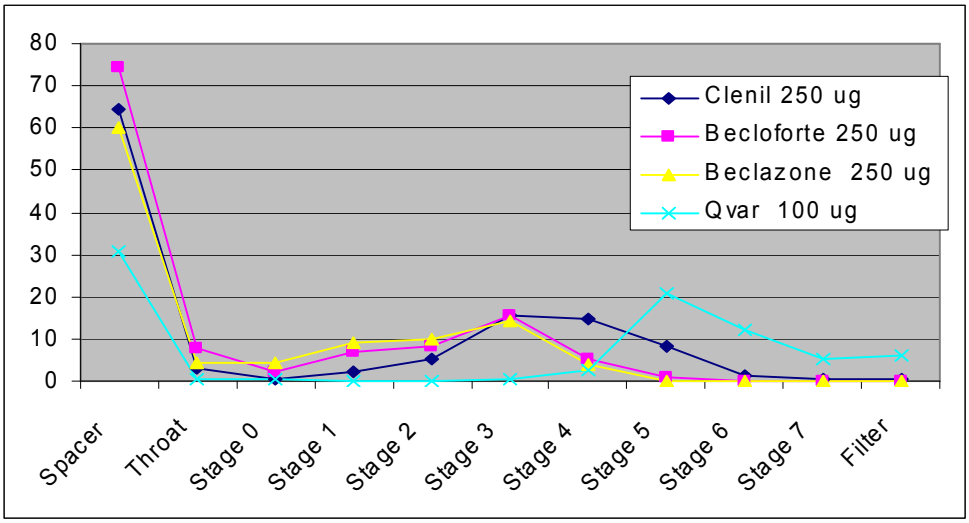

Figure 5.16 A comparison of the percentage of beclomethasone deposited on each stage of the ACI from the different pMDIs with APLUS.

Table 5.18 A comparison of cumulative mass percentages under size of beclomethasone pMDIs with APLUS deposited on each stage of the ACI.

Stage	Clenil	Becloforte	Beclazone	Qvar
Stage 0 [%]	100.00	100.00	100.00	100.00
Stage 1 [%]	99.51	94.24	89.86	98.67
Stage 2 [%]	94.75	76.24	68.63	98.56
Stage 3 [%]	84.22	54.91	44.42	98.41
Stage 4 [%]	52.41	15.74	9.99	97.87
Stage 5 [%]	22.05	2.06	0.20	92.38
Stage 6 [%]	4.71	0.07	0.02	48.89
Stage 7 [%]	1.78	0.01	0.01	23.57
Filter [%]	1.22	0.00	0.01	12.59

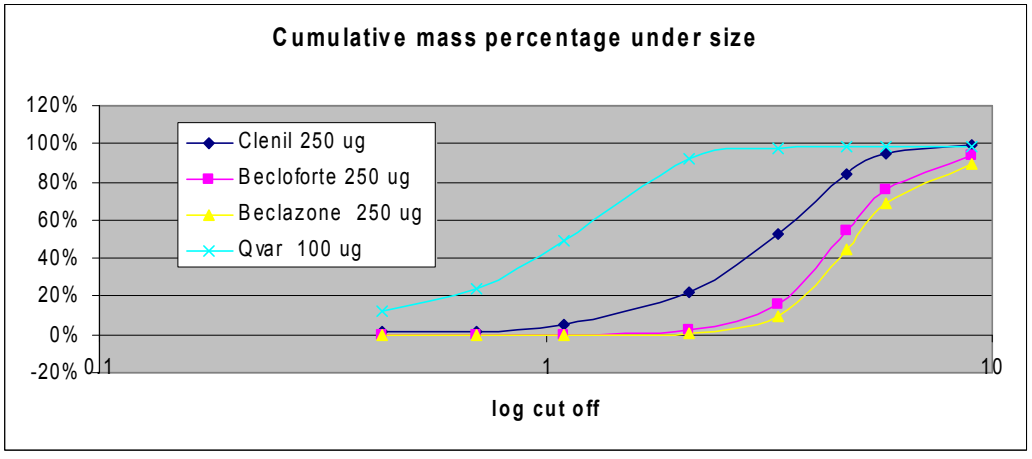

Figure 5.17 A comparison of cumulative mass percentages under size for beclomethasone pMDIs with APLUS deposited on each stage of the ACI.

Table 5.19 and Figure 5.18 show the MMAD results. The largest MMAD was Beclazone alone 5.8 μm and the smallest Qvar with APLUS 1.1 μm. There were no statistically significant differences between the pMDIs alone and pMDIs with spacers apart from Beclazone with APLUS where the difference was statistically significant ($p < 0.05$) Table 5.20. However, the differences between brand names were statistically significant ($p < 0.001$) except in the case of Becloforte and Beclazone where the difference was not statistically significant Table 5.21. Also, the effect of spacer type was not statistically significant Tables 5.22, 5.23, 5.24.

An important conclusion is that, the results of this study disagree with those reported by Barry and co-workers (1996) where they showed that, in most cases, a reduction in the size of drug particles delivered, which was demonstrated by the decrease in MMAD of the aerosol from pMDIs with spacers. However, Rahmatalla and co-workers (2002) reported no significant difference ($p < 0.1$) in MMAD after cascade impactor measurements at an inhalation flow rate of 28.3 L/min with and without a spacer.

Another study, which compared many parameters including MMAD of Flovent® CFC delivered via APLUS or Easivent® spacers versus the pMDI alone has shown no difference in MMAD (Asmus et al., 2002). Also, Cripps and co-workers (2000) examined the effect of Volumatic® and Babyhaler® spacers on the particle size distributions for the corresponding HFA 134a and CFC salbutamol and fluticasone propionate pMDIs and found no significant effect on MMAD.

The differences between brand names is influenced by a variety of factors, including chemical and physical characteristics of the propellant and the other

additives; pressure inside the canister; metering valve and actuator design; drug concentration; delivered volume and actuator and delivery outlet cleanness (Terzano, 2001). Also, the addition of non-volatile co-solvent increases the MMAD, e.g. polyethylene glycol (Ganderton et al., 2002). Furthermore, HFAs are delivered at a higher velocity than CFCs, due to a higher pressure inside its canister compared to CFCs. For this reason, metering valves using HFAs have been redesigned to separate a volume of 25 μl rather than 63 μl when CFCs are used. This alteration can be the cause of differences in particle size distribution (Terzano, 2001).

Table 5.19 MMAD values (μm) interpolated from size distribution data for different brand names of beclomethasone alone and with different spacers.

SPACER	ALONE	AMAX	OPTIMIZER	APLUS
Clenil 250 ug	3.01	3.48	3.25	3.18
Becloforte 250 ug	5.15	4.69	4.97	4.50
Beclazone Easi-Breath 250 ug	5.88	5.08	5.49	4.94
Qvar Esi-Breath 100 ug	1.31	1.43	1.17	1.11

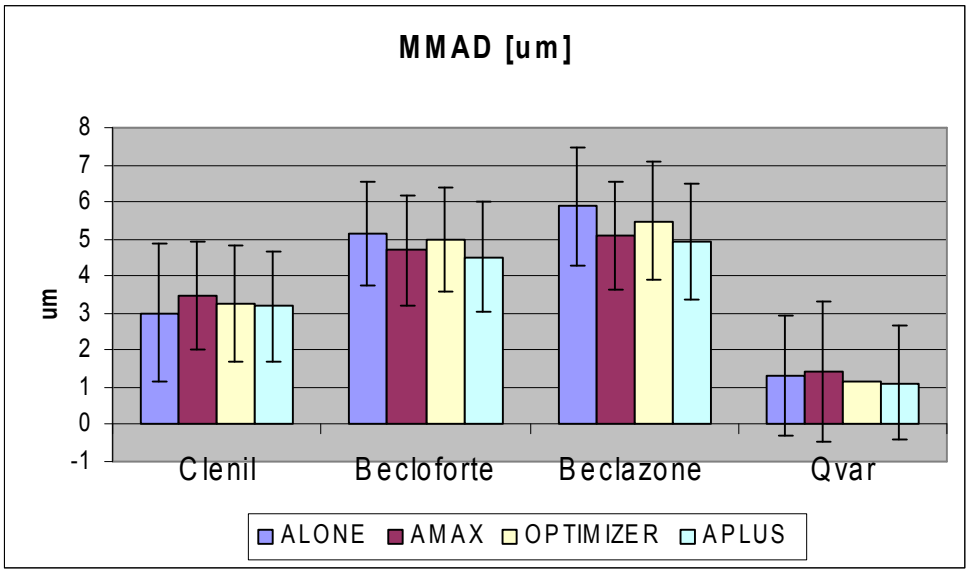

Figure 5.18 MMAD values (μm) for different brand names of beclomethasone alone and with different spacers.

Table 5.20 Mean difference for MMAD (95% confidence interval) for pMDI compared to pMDI+ spacers

	APLUS	AMAX	Optimize
Clenil®	-0.15 -0.7-0.4	-0.47 -1.0-0.1	-0.30 -0.90-0.31
Becloforte®	0.6 0.0- 1.2	0.4 -0.3- 1.0	0.2 -0.5- 0.8
Beclazone®	0.7* 0.0- 1.4	0.6 0.0- 1.3	0.2 -0.5- 0.8
Qvar®	0.2 -0.5- 0.8	-0.1 -0.8- 0.5	0.1 -0.5- 0.8

* p<0.05, ** < 0.01, ***<0.001, otherwise no significant difference.

Table 5.21 Mean difference for MMAD (95% confidence interval) between pMDIs alone.

	Clenil®	Becloforte®	Beclazone®	Qvar®
Clenil®	– – – –	2.2*** 1.6- 2.8	2.732*** 2.1- 3.3	-1.6*** -2.2- -1.0
Becloforte®	-2.2*** -2.8- -1.6	– – – –	0.5 -0.1- 1.2	-3.8*** -4.5- -3.2
Beclazone®	-2.7*** -3.3- -2.1	-0.5 -1.2- 0.1	– – – –	-4.4*** -5.0- -3.7
Qvar®	1.6*** 1.0- 2.2	3.8*** 3.2- 4.5	4.4*** 3.7- 5.0	– – – –

* p<0.05, ** < 0.01, ***<0.001, otherwise no significant difference.

Table 5.22 Mean difference for MMAD (95% confidence interval) for pMDI +AMAX compared to the pMDI+ other spacers

	APLUS	Optimize
Clenil®	0.3 -0.2- 0.8	0.2 -0.3- 0.8
Becloforte®	0.3 -0.3- 0.9	-0.1 -0.7- 0.5
Beclazone®	0.1 -0.5- 0.8	-0.4 -1.0- 0.2
Qvar®	0.3 -0.3- 0.9	0.3 -0.4- 0.9

* p<0.05, ** < 0.01, ***<0.001, otherwise no significant difference.

Table 5.23 Mean difference for MMAD (95% confidence interval) for pMDI +Optimizer® compared to pMDI+ other spacers

	AMAX	APLUS
Clenil®	-0.2 -0.8- 0.3	0.1 -0.5- 0.7
Becloforte®	0.1 -0.5- 0.7	0.4 -0.2- 1.1
Beclazone®	0.4 -0.2- 1.0	0.5 -0.1- 1.2
Qvar®	-0.3 -0.9- 0.4	0.1 -0.6- 0.7

* p<0.05, ** < 0.01, ***<0.001, otherwise no significant difference.

Table 5.24 Mean difference for MMAD (95% confidence interval) for pMDI +APLUS compared to pMDI+ other spacers

	AMAX	Optimizer
Clenil®	-0.3 -0.8- 0.2	-0.1 -0.7- 0.5
Becloforte®	-0.3 -0.9- 0.3	-0.4 -1.1- 0.2
Beclazone®	-0.1 -0.8- 0.5	-0.5 -1.2- 0.1
Qvar®	-0.3 -0.9- 0.3	-0.1 -0.7- 0.6

* p<0.05, ** < 0.01, ***<0.001, otherwise no significant difference.

Table 5.25 and Figures 5.19 and 5.20 show the throat deposition results. The highest was Becloforte alone 81.68% and the lowest was Qvar with APLUS 0. 33%. The differences between the pMDIs alone and pMDIs with spacers were statistically significant (p < 0.001) Table 5.26. Also, there were statistically significant differences between the beclomethasone pMDIs (p < 0.001) except between Clenil and Beclazone Table 5.27. Furthermore, the highest throat depositions for pMDI with spacers were for AMAX with all pMADs except Qvar Tables 5.25. Table 5.28 to Table 5.30 show the mean differences between

spacer types. The differences were statistically significant between AMAX and APLUS in case of Clenil and Beclazone and between AMAX and Optimizer in case of Becloforte and Beclazone. These findings are consistent with previous studies. The average reduction caused by spacers is 54% with range from 26% to 74%. The amount of drug deposited in the throat in this study was similar to several experiments. Bisgaard and co-workers (2002) reported the deposition with the pMDI alone ranged from 30% to 70% compared with 5% to 10% with spacers. Rahmtalla and co-workers (2002) examined the effect of spacer on the mouth-throat deposition of Qvar and found that adding the spacer reduced drug deposition in the throat. Asmus and co-workers (2002) tested the performance of spacers with a fluticasone pMDI. Their results showed a decrease in quantity of drug deposited in the throat so they suggested the use of spacers may diminish the risk of topical adverse effects. Spacers reduce deposition in the mouth and throat, decreasing cough, and also may decrease oral candidiasis when oral inhaled corticosteroids are used. Furthermore, their use may decrease the systemic bioavailability and the risk of systemic side effects (Rabe et al., 2007). Also, radio-labelling data for Qvar showed an up to three time lower dose is deposited in the throat when the spacer is used (Bisgaard et al., 2002). In addition, Roland and co-workers (2004) suggested the spacers use as a part of treatment to prevent local side effects recurring through reduction of throat deposition. However, another study found that the spacer may increase the incidence of cough.

Table 5.25 Throat deposition (%) of nominal dose for different brand names of beclomethasone alone and with different spacers

	ALONE	AMAX	Optimizer	APLUS
Clenil®	67.52	10.10	3.87	3.21
Becloforte®	81.68	13.92	6.32	7.79
Beclazone®	64.96	12.80	3.48	4.34
Qvar®	28.67	2.72	3.61	0.33

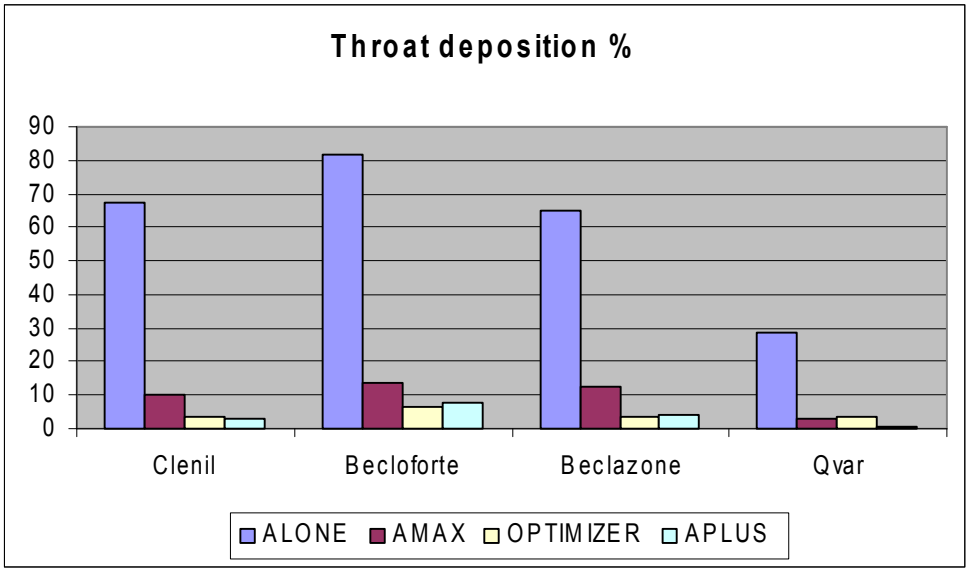

Figure 5.19 Throat deposition (%) of nominal dose for different brand names of beclomethasone alone and with different spacers.

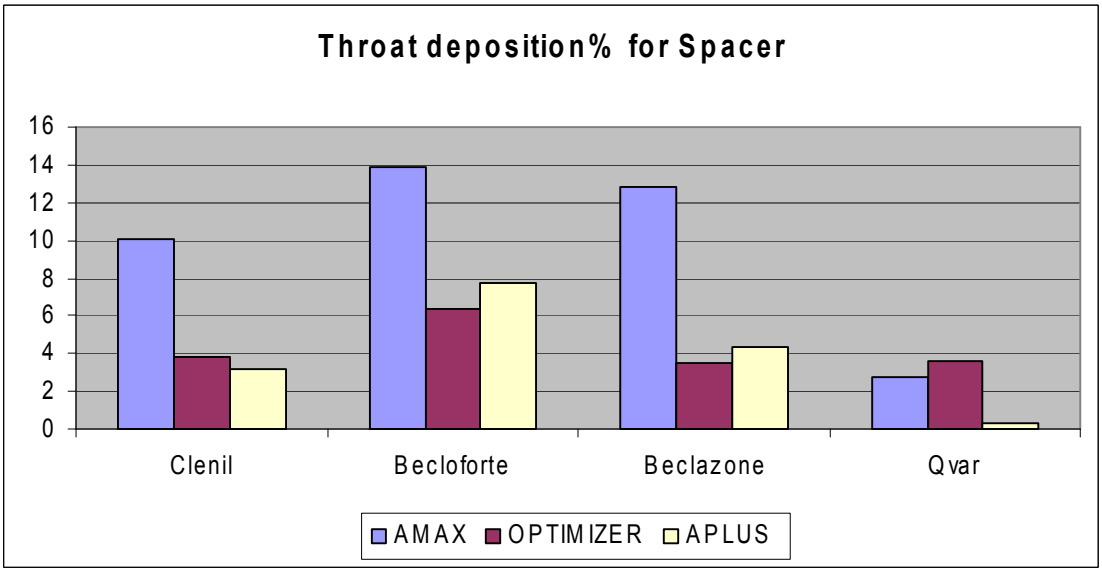

Figure 5.20 Throat deposition (%) of nominal dose for different brand names of Beclomethasone with different spacers.

Table 5.26 Mean difference for throat deposition (95% confidence interval) for pMDI compared to pMDI+ Spacers

	APLUS	AMAX	Optimize
Clenil®	64.3*** 58.2- 70.5	57.4*** 51.3- 63.6	63.7*** 56.5- 70.8
Becloforte®	73.897*** 66.0- 81.8	67.763*** 60.3- 75.2	75.363*** 67.4- 83.3
Beclazone®	60.6*** 52.7- 68.6	52.2*** 44.2- 60.1	61.5*** 53.5- 69.4
Qvar®	28.3*** 20.4- 36.3	26.0*** 17.8- 34.1	25.1*** 16.9- 33.2

* p<0.05, ** < 0.01, ***<0.001, otherwise no significant difference.

Table 5.27 Mean difference for throat deposition (95% confidence interval) between pMDIs

	Clenil®	Becloforte®	Beclazone®	Qvar®
Clenil®	----	14.2*** 7.1- 21.3	-2.6 -9.7- 4.6	-38.9** -46.0- -31.7
Becloforte®	-14.2*** -21.3- -7.1	----	-16.7*** -24.7- -8.8	-53.0*** -61.0- -45.1
Beclazone®	2.6 -4.6- 9.7	16.7*** 8.8- 24.7	----	-36.3*** -44.2- -28.4
Qvar®	38.9*** 31.7- 46.0	53.0*** 45.1- 61.0	36.300*** 28.4- 44.2	----

* p<0.05, ** < 0.01, ***<0.001, otherwise no significant difference.

Table 5.28 Mean difference for throat deposition (95% confidence interval) for pMDI +AMAX compared to pMDI+ other spacers

	APLUS	Optimize
Clenil®	6.9* 0.6- 13.2	7.0 -0.5- 14.6
Becloforte®	6.5 -1.3- 14.3	8.0* 0.2- 15.7
Beclazone®	8.5* 0.3- 16.6	9.3* 1.2- 17.5
Qvar®	2.4 -5.8- 10.5	-0.9 -9.1- 7.3

* p<0.05, ** < 0.01, ***<0.001, otherwise no significant difference.

Table 5.29 Mean difference for throat deposition (95% confidence interval) for pMDI +Optimizer® compared to pMDI+ other spacers

	AMAX	APLUS
Clenil®	-7.0 -14.6- 0.5	-0.1 -7.7- 7.4
Becloforte®	-8.0* -15.7- -0.2	-1.5 -9.6- 6.7
Beclazone®	-9.3* -17.5- -1.2	-0.9 -9.0- 7.3
Qvar®	0.9 -7.3- 9.1	3.3 -4.9- 11.4

* p<0.05, ** < 0.01, ***<0.001, otherwise no significant difference.

Table 5.30 Mean difference for throat deposition (95% confidence interval) for pMDI +APLUS compared to pMDI+ other spacers

	AMAX	Optimizer
Clenil®	-6.9* -13.2- -0.6	0.1 -7.4- 7.7
Becloforte®	-6.5 -14.3- 1.3	1.5 -6.7- 9.6
Beclazone®	-8.5* -16.6- -0.3	0.9 -7.3- 9.0
Qvar®	-2.4 -10.5- 5.8	-3.3 -11.4- 4.9

* p<0.05, ** < 0.01, ***<0.001, otherwise no significant difference.

Table 5-31 and Figure 5-21 illustrates the FPD results. The highest FPD was Qvar with AMAX 64% while the lowest FPD was Beclazone alone. In addition, the effect of spacers, on the FPD with CFC pMDIs; was not statistically significant however, in the case of CFC-free pMDIs, the effect of spacers depended on spacer type. The effect of APLUS on FPD was statistically significant, while the effect of the optimizer was not statistically significant. On the other hand, the effect of AMAX was significant only with Clenil ($p < 0.001$) Table 5-32. Table 5-33 illustrates the differences among pMDIs. Qvar

delivered a statistically significant FPD comparing to other pMDIs. Tables 5.34 – 5.36 summarise the statistical test result for differences between spacers for FPD. Since the spacers produced an FPD not significantly different from that of the pMDI alone for CFC pMDI. It may suggest that any of these spacers would be equally effective for pMDI alone with an appropriate inhaler technique.

Rahmatalla and co-workers (2002) showed a selective effect for spacers, reducing the throat deposition while slightly increasing the lung deposition. However there was no significant influence on the size distribution of FPD after examining the effect of a spacer on Qvar aerodynamic characterisation. Also, Leach and co-workers (1998) found no significant differences in lung deposition when a spacer was used *in-vivo;* however, there was a large variability in *in-vivo* results. In addition, Bisgard and co-workers (2002) reported that the lung dose with intermediate and large volume spacers is about double the dose compared to pMDI alone; however, in other studies, the large and small volume spacers delivered a lung dose similar to pMDI alone.

In contrast, Fink and co-workers (1998) compared the effect of several spacers on salbutamol pMDI. They found a significant variation in FPD when compared to pMDI alone. FPD was similar for the Aerochamber®, but there was a 33%, 35% and 55% reduction for Optihaler®, Ace®, and Inspirease®, respectively. Furthermore, another study compared Flovent® CFC delivery with APLUS and Optichamber® spacer to pMDI alone in terms of FPD and showed equivalent delivery (Asmus et al., 2002).

Use of ethanol to reformulate Qvar resulted in a an increase in the FPD which led to a two-fold reduction in dosage with the Qvar compared with the CFC pMDI under certain conditions (Cripps et al., 2000).

The spacers allow more time for the propellant to evaporate; this promotes the formation of small aerosol particles 1-5 μm which are more likely to be entrained by inspiration into small human airways (Asmus et al., 2004). In addition, the spacer acts as a settling chamber, allowing large particles to sediment or impact. Therefore the final size of drug particles depends on the time available for evaporation of propellant and distance from the actuator orifice (Bisgaard et al., 2002). Faarc and co-workers (2006) studied a nonelectrostatic versus a non-conducting spacer using Xopenex® (Levalbuterol) HFA Inhalation, the FPD difference between AMAX and APLUS was statistically significant in their study. Furthermore, Rau and co-workers (2006) reported that electrostatic charge is more prevalent with HFA formulations compared to CFC. Moreover, the half life of the aerosol inside the spacer is reduced by electrostatic activity of the spacer (Bisgaard et al., 2002). Terzano (2001) concluded that non-electrostatic spacers delivered a significantly higher dose than non-conducting; furthermore, a reduction in dose should be considered when CFC-free formulation is used with a spacer.

Table 5.31 FPD (%) of nominal dose for different brand names of beclomethasone alone and with different spacers

	ALONE	AMAX	OPTIMIZER	APLUS
Clenil®	25.55	50.13	25.03	42.70
Becloforte®	18.71	26.95	18.84	24.33
Beclazone®	17.08	21.84	18.06	21.57
Qvar®	59.60	64.30	53.97	47.26

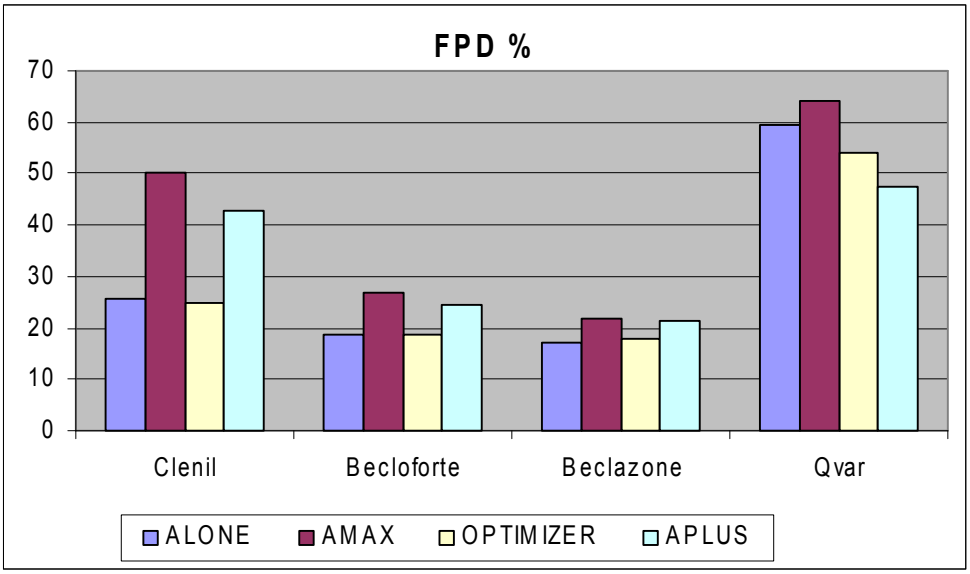

Figure 5.21 FPD (%) of nominal dose for different brand names of Beclomethasone alone and with different spacers

Table 5.32 Mean difference for FPD (95% confidence interval) for pMDI alone compared to pMDI+ spacers

	APLUS	AMAX	Optimize
Clenil®	-17.2*** -24.7- -9.6	-24.6*** -32.1- -17.1	0.5 -8.1- 9.2
Becloforte®	-5.6 -15.3- 4.1	-8.3 -17.3- 0.8	-0.1 -9.8- 9.6
Beclazone®	-4.2 -13.8- 5.5	-4.4 -14.1- 5.3	-0.6 -10.3- 9.1
Qvar®	12.5*** 2.8- 22.2	-4.5 -14.2- 5.2	5.4 -4.3- 15.1

* p<0.05, ** < 0.01, ***<0.001, otherwise no significant difference.

Table 5.33 Mean difference for FPD (95% confidence interval) between pMDIs

	Clenil®	Becloforte®	Beclazone®	Qvar®
Clenil®	– – – –	-6.8 -15.5- 1.8	-8.1 -16.8- 0.5	34.0*** 25.4- 42.7
Becloforte®	6.8 -1.8- 15.5	– – – –	-1.3 -11.0- 8.4	40.9*** 31.2- 50.6
Beclazone®	8.1 -0.5- 16.8	1.3 -8.4- 11.0	– – – –	42.2*** 32.5- 51.9
Qvar®	-34.0*** -42.7 -25.4	-40.9*** -50.6- -31.2	-42.2*** -51.9- -32.5	– – – –

* p<0.05, ** < 0.01, ***<0.001, otherwise no significant difference.

Table 5.34 Mean difference for FPD (95% confidence interval) for pMDI + AMAX compared to pMDI+ other spacers

	APLUS	Optimize
Clenil®	7.4 -0.1- 14.9	25.1*** 16.4- 33.8
Becloforte®	5.4 -3.7- 14.4	8.1 -0.9- 17.2
Beclazone®	-2.5 -12.1- 7.2	3.8 -5.9- 13.5
Qvar®	17.0** 7.3- 26.7	9.9* 0.2- 19.6

* p<0.05, ** < 0.01, ***<0.001, otherwise no significant difference.

Table 5.35 Mean difference for FPD (95% confidence interval) for pMDI + Optimizer® compared to pMDI+ other spacers

	AMAX	APLUS
Clenil®	-25.1*** -33.8- -16.4	-17.7*** -26.3- -9.0
Becloforte®	-8.1 -17.2- 0.9	-5.5 -15.2- 4.2
Beclazone®	-3.8 -13.5- 5.9	-3.5 -13.2- 6.2
Qvar®	-9.9* -19.6- -0.2	7.1 -2.6- 16.7

* p<0.05, ** < 0.01, ***<0.001, otherwise no significant difference.

Table 5.36 Mean difference for FPD (95% confidence interval) for pMDI + APLUS compared to pMDI+ other spacers

	AMAX	Optimizer
Clenil®	-7.4 -14.9- 0.1	17.7*** 9.0- 26.3
Becloforte®	-2.7 -11.7- 6.4	5.5 -4.2- 15.2
Beclazone®	-0.3 -10.0- 9.4	3.5 -6.2- 13.2
Qvar®	-17.0** -26.7- -7.3	-7.1 -16.7- 2.6

* p<0.05, ** < 0.01, ***<0.001, otherwise no significant difference.

Tables 5.37 and Figure 5.22 demonstrate the FPF results. The FPF is calculated by equation 3.1. The highest FPF was Qvar with APLUS and the lowest was with Becloforte alone. There were strong statistically significant differences between pMDIs alone and pMDIs with spacers ($p < 0.001$) Table 5.38. As the spacer retains large particles and passes the small, the equation to calculate FPF is

$$\text{FPF} = \frac{R}{\sum A}$$

Where ∑A is delivered dose, R is FPD

Therefore the FPF result with spacer is higher since the delivered dose is smaller than pMDI alone.

The differences among the pMDIs alone were statistically significant except between Beclazone and Becloforte Table 5.39. There were significant differences between APLUS and AMAX in Clenil, Becloforte and Beclazone, also between APLUS and Optimizer in Clenil, Becloforte and Beclazone, however the differences between the Optimizer and AMAX were not statistically significant; this may be explained by the amount retained in the AMAX and Optimizer being smaller because AMAX is non-electrostatic and Optimizer is a small volume spacer (50 ml) while the APLUS is a non-conducting spacer with a volume of 149 ml Tables 5.40 –5.42.

Table 5.37 FPF for different brand names of beclomethasone alone and with different spacers.

	ALONE	AMAX	OPTIMIZER	APLUS
Clenil®	25.93	70.50	69.94	82.61
Becloforte®	15.45	42.05	43.28	51.29
Beclazone®	16.06	37.27	37.22	46.52
Qvar®	66.49	90.79	92.25	97.11

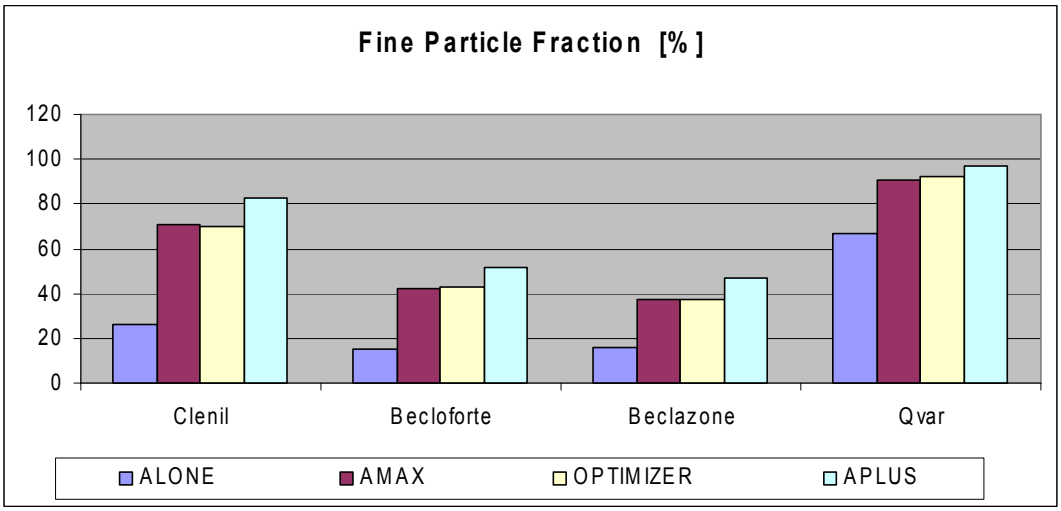

Figure 5.22 FPF for different brand names of Beclomethasone alone and with different spacers.

Table 5.38 Mean difference for FPF % (95% confidence interval) for pMDI alone compared to pMDI+ spacers

	APLUS	AMAX	Optimize
Clenil®	-56.7*** -62.1- -51.3	-44.6*** -50.0- -39.1	-44.0*** -50.3- -37.8
Becloforte®	-35.8*** -42.8- -28.9	-26.6*** -33.1- -20.1	-27.8*** -34.8- -20.8
Beclazone®	-30.5*** -37.5- -23.5	-21.2*** -28.2- -14.2	-21.1*** -28.2- -14.2
Qvar®	-30.6*** -37.6- -23.6	-24.3*** -31.3- -17.3	-25.8*** -32.8- -18.8

* p<0.05, ** < 0.01, ***<0.001, otherwise no significant difference.

Table 5.39 Mean difference for FPD (95% confidence interval) between pMDIs

	Clenil®	Becloforte®	Beclazone®	Qvar®
Clenil®	----	-10.5** -16.7- -4.2	-9.9** -16.1- -3.6	40.6*** 34.3- 46.8
Becloforte®	10.5** 4.2-16.7	----	0.6 -6.4- 7.6	51.0*** 44.0- 58.0
Beclazone®	9.9** 3.6- 16.1	-0.6 -7.6- 6.4	----	50.4*** 43.4- 57.4
Qvar®	-40.6*** -46.8- -34.3	-51.0*** -58.0- -44.0	-50.4*** -57.4- -43.4	----

* p<0.05, ** < 0.01, ***<0.001, otherwise no significant difference.

Table 5.40 Mean difference for FPF (95% confidence interval) for pMDI + AMAX compared to pMDI+ other spacers

	APLUS	Optimize
Clenil®	-12.1*** -17.5 -6.7	0.6 -5.7- 6.8
Becloforte®	-9.2* -15.8 -2.7	-1.2 -7.8- 5.3
Beclazone®	-9.2* -16.2- -2.3	0.1 -6.9- 7.0
Qvar®	-6.3 -13.3- 0.7	-1.5 -8.5- 5.5

* p<0.05, ** < 0.01, ***<0.001, otherwise no significant difference.

Table 5.41 Mean difference for FPF (95% confidence interval) for pMDI + Optimizer® compared to pMDI+ other spacers

	AMAX	APLUS
Clenil®	-0.6 -6.8- 5.7	-12.7*** -18.9- -6.4
Becloforte®	1.2 -5.3- 7.8	-8.0* -15.0- -1.0
Beclazone®	-0.1 -7.0- 6.9	-9.3* -16.3- -2.3
Qvar®	1.5 -5.5- 8.5	-4.9 -11.8- 2.1

* p<0.05, ** < 0.01, ***<0.001, otherwise no significant difference.

Table 5.42 Mean difference for FPF (95% confidence interval) for pMDI + APLUS compared to pMDI+ other spacers.

	AMAX	Optimizer
Clenil®	12.1*** 6.7- 17.5	12.7*** 6.4- 18.9
Becloforte®	9.2* 2.7- 15.8	8.0* 1.0- 15.0
Beclazone®	9.3* 2.3- 16.2	9.3* 2.3- 16.3
Qvar®	6.3 -0.7- 13.3	4.9 -2.1- 11.8

* p<0.05, ** < 0.01, ***<0.001, otherwise no significant difference.

CONCLUSION

The MMAD is not affected when a spacer is used with the pMDI when compared to its use alone, but there are significant differences between pMDIs. In contrast with the pMDI alone, spacers, especially the APLUS, markedly reduces throat drug deposition. The reduction takes place because spacers have a size-selective function, retaining larger particles on the spacer and allowing smaller particles to pass. Therefore, a proportion of the particles that would have been deposited in the throat is shifted to the spacer itself and thereby diminishes the risk of local adverse effects. In addition, compared with the pMDI alone, the FPD is generally either increased or unaffected by using spacers. Several factors can cause these variable findings, for instance the electrostatic charge present in the spacer, as well as the volume. There are strong statistically differences between pMDIs alone and pMDIs with spacers in FPF which can be explained by the reduction in total delivered dose since the spacer retains a part of the emitted dose.

Future works

A principal limitation of the study is that the ACI is based on sampling the aerosol at a constant flow rate and does not reflect patient-specific variables such as lung volume, lung function and breathing pattern. Future works should include measurement of aerosol deposition under more physiological conditions, particularly those likely to be encountered in patients with asthma and COPD. Furthermore, a clinical study is needed to confirm if FPD differences among these pMDIs translate to significant clinical differences.

Another limitation is that the study results only reflect the performance of these spacers with the Beclomethasone pMDI formulations containing CFC

and CFC-free propellants. They may not be reflective of performance with other brands of the same drug or of different pMDIs. The study results suggest that it is inappropriate to use a drug with any device without evaluating the interaction between pMDIs and the spacer with which they are used.

The study showed a significant reduction in throat drug deposition with the pMDI alone, comparing to pMDI with spacer. It would be considerable advantage if spacer is tested with DPIs to reduce the throat deposition.

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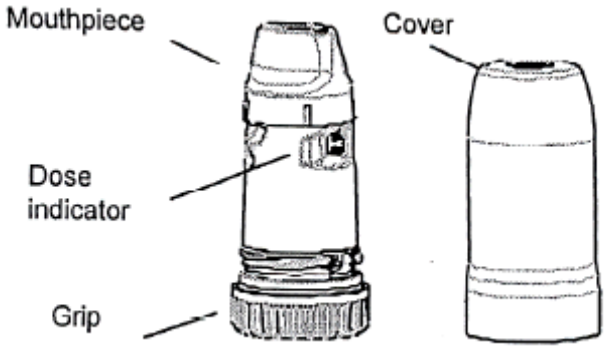
APPENDICES

APPENDICES

APPENDIX A PATIENT INFORMATION LEAFLETS

How to prepare a new inhaler for use
Before you start using SYMBICORT TURBUHALER for the first time it is important that you read the instructions below and follow them carefully.
TURBUHALER is an inhaler from which very small amounts of powder are administered. When you breathe in through TURBUHALER the powder is delivered to the lungs. It is therefore important that you inhale forcefully and deeply through
the mouthpiece.



SYMBICORT TURBUHALER is very easy to use. If you follow the instructions below, you will receive the' medication.
Preparation before first use of new inhaler:
Before using your inhaler for the first time you need to prepare the inhaler for use. The preparation does not need to be repeated even if your inhaler is not used regularly.
1. Unscrew and lift off the cover. You may hear a rattling sound. This is normal.
2. Hold the inhaler upright with the red grip downwards Fig.1). Turn the red grip as far as it will go in one direction and then turn it back as far as it will go in the opposite direction. Perform this procedure twice.

Figure 1

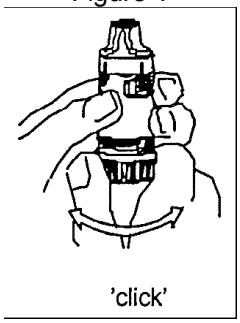
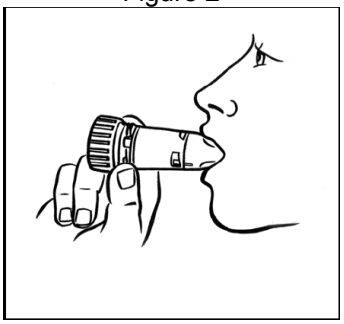


Figure 2

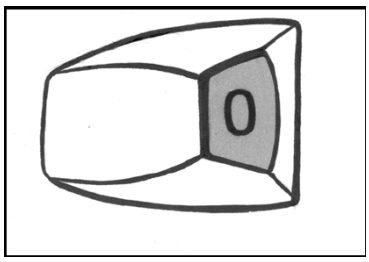


B. Using the inhaler
To administer a dose, simply follow the instructions below. Unscrew and lift off the cover. A rattling sound may be heard. This is normal.
TURN
Hold the inhaler upright with the red grip downwards Fig. 1). Do not hold the mouthpiece when turning the grip. To load the inhaler with a dose turn the grip as far as it will go in one direction and then turn it back again as far as it will go in the opposite direction You will hear a 'click' some time during this procedure. The inhaler is now ready to use. Breathe out. Do not breathe out through the mouthpiece.
INHALE
Place the mouthpiece gently between your teeth. Close your lips and breathe in forcefully and deeply through your mouth Fig. 2). You may not taste or feel any medication when inhaling.

This is common. Do not chew or bite on the mouthpiece.
Remove the inhaler from your mouth before breathing out again.
If more than one dose has been prescribed, repeat the steps above.
Replace the cover.
If you accidentally drop, shake or breathe out into SYMBICORT TURBUHALER after it is loaded, you will lose your dose. If this happens, you should load a new dose and inhale it.
Note: Never breathe out through the mouthpiece. Always replace the cover properly after use. Do not try to remove the mouthpiece or to twist it unnecessarily; it is fixed to the inhaler and must not be taken off. As the amount of powder dispensed is very small, you may not be able to taste or feel it after inhalation. However, you can still be confident that the dose has been inhaled if you have followed the instructions.

Stopping SYMBICORT TURBUHALER

Talk to your doctor about how to gradually stop your medication if necessary. It is important that you do not suddenly stop taking SYMBICORT TURBUHALER as it may cause unwanted side effects.
Cleaning: Clean the outside of the mouthpiece once a week with a dry tissue. Never use water or any other fluid when cleaning the mouthpiece. If fluid enters the inhaler it may not work properly.
When to start a new inhaler: SYMBICORT TURBUHALER has a dose indicator. The dose indicator tells you how many doses are left in the inhaler. The dose indicator moves slowly each time you load a dose. Every 20th dose is marked with a number and every 10th dose is marked with a dash. When the "0" on the red background has reached the middle of the window you should discard your inhaler. The sound you hear when you shake the inhaler is produced by a drying agent, not the medication. SYMBICORT TURBUHALER cannot be re-filled with drug and should be discarded.



Dose indicator shows that it is time to start a new inhaler

Symbicort patient information leaflet

Instructions for use:
It is important that you know how to use your inhaler properly. Your doctor, nurse or pharmacist will show you how to use your inhaler correctly and will check regularly that you are using your inhaler correctly. You must follow their instructions carefully, so that you know how, when and how many puffs to inhale and how often you must use your inhaler. The instructions should be on the pharmacist's label and are given in this leaflet. If you are not sure what to do or have problems inhaling then ask your doctor, nurse or pharmacist for advice.

To remove the mouthpiece cover, hold between the thumb and forefinger, squeeze gently and pull apart as shown. Check inside and outside to make sure that the mouthpiece is clean, and that there are no foreign objects. Testing Your Inhaler: If the inhaler is new or if it has not been used for three days or more, one puff should be released into the air to make sure that it works.
Hold the inhaler upright as shown, with your thumb on the base, below the mouthpiece.
Breathe out as far as is comfortable, place the mouthpiece in your mouth between your teeth and close your lips around it but do not bite it.
Just after starting to breathe in through your mouth press down on the top of the inhaler to release a puff while still breathing in steadily and deeply.
Hold your breath; take the inhaler from your mouth and your finger from the top of the inhaler.
Continue holding your breath for a few seconds or as long as is comfortable. Breathe out slowly.
If you are to take another puff, keep the inhaler upright and wait about half a minute before repeating steps 2 to 5**.**
After use always replace the mouthpiece cover to keep out dust and fluff. Replace firmly and snap into position.
Important: Do not rush steps 2, 3, 4 and 5.
Cleaning:
It is important to clean your inhaler at least once a week, to stop it blocking up.
Pull the metal canister out of the plastic case of the inhaler and remove the mouthpiece cover.
Rinse the plastic case and the mouthpiece cover in warm water. If you use a mild liquid detergent, rinse carefully with clean water before drying. Do not put the metal canister into water.
Leave to dry thoroughly in a warm place. Avoid excessive heat.
Replace the canister and mouthpiece cover.
It is important that you also read the Package leaflet which is supplied with your Volumatic spacer device and that you follow the instructions on how to use the Volumatic and on how to clean it, carefully.

Clenil Patient Information Leaflet

PATIENT'S INSTRUCTIONS
It is important that you read these instructions before using QVAR.
Correct and regular use of the inhaler will prevent or lessen the severity of asthma attacks.
Remove the plastic cap see Figure 1) and be sure there are no foreign objects in the mouthpiece.

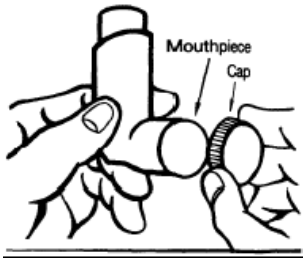


Figure 1
As with all aerosol medications, it is recommended to prime the QVAR inhaler before using for the very first time after purchase, and in cases where the inhaler has not been used for more than ten days. Prime by releasing two actuations into the air, away from your eyes and face. Be sure the canister is firmly seated in the plastic mouthpiece adapter before each use.
BREATHE OUT AS FULLY AS YOU COMFORTABLY CAN. Hold the inhaler as shown in Figure 2. Close your lips around the mouthpiece, keeping your tongue below it.

Figure 2
WHILE BREATHING IN DEEPLY AND SLOWLY, PRESS DOWN ON THE CAN WITH YOUR FINGER. When you have finished breathing in, hold your breath as long as you comfortably can i.e., 5 – 10 seconds).
TAKE YOUR FINGER OFF THE CAN and remove the inhaler from your mouth. Breathe out gently.
IF YOUR PHYSICIAN HAS TOLD YOU TO TAKE MORE THAN ONE INHALATION PER TREATMENT REPEAT STEPS 3 THROUGH 5.
You should rinse your mouth with water after treatment.
For normal hygiene, the mouthpiece of your inhaler should be cleaned weekly with a clean, dry tissue or cloth. DO NOT WASH OR PUT ANY PART OF YOUR INHALER IN WATER.

Qvar aerosol Patient Information Leaflet

How to use your Beclazone Easi-Breathe Inhaler

Beclazone Easi-Breathe inhaler is designed to make it easier for you to take your medicine. It is a breath-actuated inhaler. This means that it only releases a actuation when you breathe in.

IMPORTANT:

Before you use your Inhaler, please read this leaflet carefully and follow the instructions,

TESTING YOUR INHALER

Test spray your inhaler before you use it for the first time and also if you have not used it for a week or more. Unscrew the top of your inhaler so that you can see the metal canister inside Open the cap, shake the Inhaler and spray the aerosol by pressing the canister with your finger or thumb, Close the cap and replace the top. Your inhaler is now ready for use.

This pack also contains a spacer called the Optimizer which can be used with your Beclazone Easi-Breathe inhaler,

Your doctor or pharmacist may have already advised you to use the Optimiser with your Inhaler. If you have been advised to use the Optimiser follow the instructions in 'How to use your inhaler with the Optimiser' B). If not, follow instructions A).

A) How to use your inhaler without the Optimiser

Shake the inhaler vigorously.

Hold the Inhaler uptight and open It by folding down the cap which fits over the mouthpiece.

Breathe out normally as Far as you comfortably can and then.

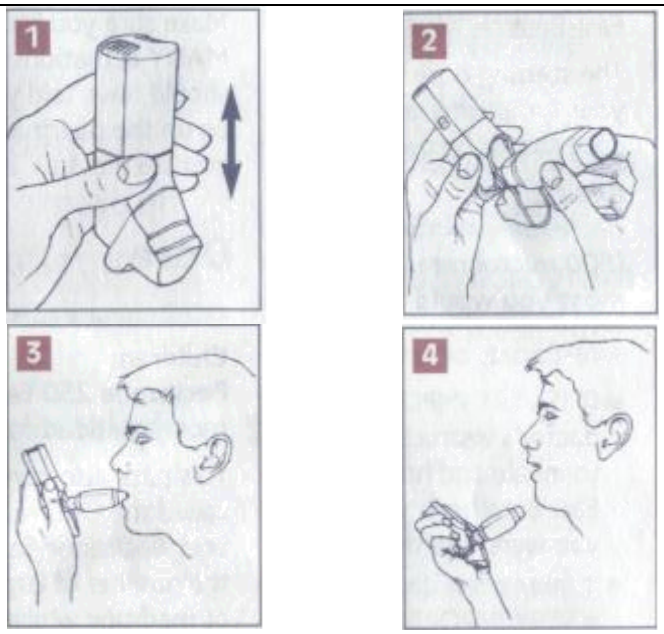
Place the mouthpiece in your mouth between your teeth and close your lips firmly around It, but do not bite it. Make sure that your hand is not blocking the airholes.

Breathe in slowly and deeply through mouthpiece. Don't stop breathing when the inhaler actuates the dose into your mouth. Carry on until you have taken a deep breath.

Take the inhaler out of your mouth and hold your breath for 10 seconds or as Long as is comfortable. Then breathe out slowly.

After you have used your inhaler, hold it upright and close the cap immediately.

If you need to take a second actuation, it is important to re-prime your inhaler by closing the cap and waiting about one minute before starting again from step 1. If your inhaler is not re-primed you will not receive a second actuation.



B) How to use your inhaler with the Optimiser

Shake the inhaler vigorously.
Hold the inhaler upright and open it by folding down the cap which fits Over the mouthpiece.
Slot the Optimiser firmly onto the mouthpiece of the inhaler.
Breathe out normally as far as you comfortably can and then.
Place the mouthpiece of the Optimizer in your mouth between your teeth and close your lips firmly around it, but do not bite it. Make sure that your hand Is not blocking the airholes.
Breathe in slowly and deeply through the Optimiser. Do not stop breathing when the inhaler actuates the dose into your mouth. Carry on until you have taken a deep breath.
Take the Optimiser out of your mouth and hold your breath for 10 seconds or as long as Is comfortable. Then breathe out slowly,
After you have used your inhaler, hold it upright, remove the Optimiser and close the cap immediately.
If you need to take a second actuation, It is important to re-prime your inhaler by closing the cap and waiting about one minute before starting again from step '1. if your inhaler is not re-primed you will not receive a second actuation.
You must keep your Inhaler clean, especially In the mouthpiece. This will prevent deposits from the aerosol building up. Wash your inhaler once a week.
in the unlikely event that your Inhaler does not work property, unscrew the top, open the cap and press the canister down, If your inhaler does not spray, It may be empty and should be replaced. If it sprays successfully, screw the top back on, close the cap and use as above.

Cleaning

Before cleaning make sure the cap is closed.
Unscrew the top of your inhaler. Keep this top dry at all times
Remove the metal canister Do not put this metal canister In water
Open the cap, rinse the inhaler body in warm water and dry it.
Put the canister back In. your inhaler, screw the top back on and close the cap.
Do not wash the top part of the inhaler.
Do not use any heat to dry the inhaler body.

Beclazone Easi-Breathe Patient Information Leaflet

HOW TO USE YOUR INHALER

1) To remove the SNAP-ON mouthpiece cover from the inhaler, hold between the thumb and finger,. squeeze gently and pull apart as shown. check inside and outside to make sure that the mouthpiece is clean and that there ,are no foreign objects.

TESTING YQUR INHALER.

If your 'inhaler is new or if it has not been used for a week or more, shake it well and release one puff into the air to make sure that it works.

2) Shake the inhaler before use.

3) Hold the inhaler upright as shown above will, your thumb on the base, below the mouthpiece, Breathe out as far as is comfortable and then…..

4) Place the mouthpiece in your mouth between your teeth arid close your lips around it but do not bite it,

5) Just after starting to breathe in through your mouth press down on the top of the inhaler to release a puff while still breathing in steadily and deeply.

6) Hold your breath, take the inhaler from your mouth and your finger from the top of the inhaler. Continue holding your breath for a few seconds or as long as is comfortable

7) If you are going to take another puff keep the inhaler upright and wait for about half a minute before repeating steps 2 to 6.

8) After use always replace the mouthpiece cover to keep out dust and fluff
REPLACE FIRMLY AND SNAP INTO POSITION.

IMPORTANT

Do not rush stages 3,4 and 5.

It is important that you start to breathe in as slowly as possible ,just before operating your inhaler. Practise in front of a mirror for the first few times. If you see mist coming from top of inhaler or the sides of your mouth you should start again from stage 2.

Some people find it difficult to release a puff of medicine just after they start to breathe in. The Volumatic large-volume spacer device helps to overcome this problem your doctor or pharmacist will be able to advise you about this.

People with weak hands may find it easier to hold the inhaler with both hands as shown. Put the two forefingers on top of the inhaler and both thumbs on the bottom below the mouthpiece.If this does not help, a special device called a Halersid$_{TM}$., may make it easier your doctor, nurse or pharmacist will he able to advise you.)
If you have been given different instructions for using your inhaler, please fallow them carefully Tell your doctor, nurse or pharmacist If you, have my difficulties.

CLEANING

Your inhaler should lie cleaned at least once a week
I. Pull the metal canister out of the plastic case of the inhaler and remove the mouthpiece cover.
2. Rinse the plastic case and the mouthpiece cover in warm water. A mild detergent or a solution of the type used to clean babies feeding bottles may he added in the water your pharmacist will advise you). Then rinse thoroughly with clean water before drying. Do not put the metal canister into water
3. Leave to dry in a warns place Avoid excessive heat
4. Replace the canister and mouthpiece cover.

Becloforte and Becotide Patient Information Leaflet

You have purchased the industry's leading Valved Holding Chamber, ***AeroChamber MAX®*** VHC.

New ***AeroChamber MAX®*** VHC virtually eliminates the guesswork associated with aerosol drug delivery through innovative features such as the ***Flow-Vu™*** Inspiratory Flow Indicator, which will greatly assist you or a caregiver when delivering your aerosol medication. The additional product enhancements, such as the Non-Static ***StatBan™*** Chamber, can improve the therapeutic benefit of the inhaled medication that your doctor prescribed.

Now you can *Breathe Easier*...with new ***AeroChamber MAX®*** VHC.

Directions for Use (Single Patient Use Only)

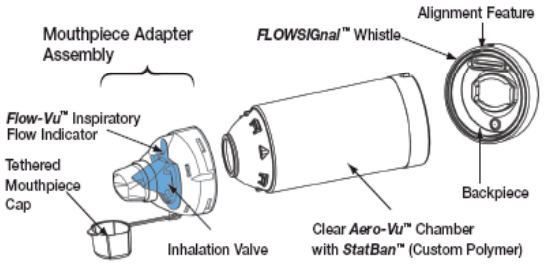
	ENGLISH
1	Prior to use, carefully examine the product for proper assembly, foreign objects, and damaged or missing parts. Ensure the Mouthpiece Adapter Assembly is properly secured to the chamber body. Remove any foreign objects prior to use. The product should be replaced IMMEDIATELY if there are any damaged or missing parts. If necessary, use the Metered Dose Inhaler (MDI) alone until a replacement is obtained. If the patient's symptoms worsen, please seek immediate medical attention.
2	Remove cap(s). Before use, ensure the instructions supplied with the MDI have been read.
3	Shake the MDI immediately before each use as per the instructions supplied with MDI. Insert the MDI into the Backpiece of the chamber.
4	Place the mouthpiece comfortably between the lips as shown. Ensure a mouth to mouthpiece seal by watching the movement of the ***Flow-Vu™*** Inspiratory Flow Indicator. As the patient inhales through the chamber, the brightly colored Flow Indicator should move towards the patient, providing visual feedback of the inhalation maneuver. The Flow Indicator will return to the vertical position when the patient stops inhalation or exhales. The exhaled breath exits the chamber as shown in the diagram.
5	Use the ***Flow-Vu™*** Flow Indicator to assist in the coordination of the inhalation maneuver. As the patient begins a slow deep inhalation, the Flow Indicator will move towards the patient. This is the correct time to depress the MDI. A breath hold of up to 10 seconds is recommended to improve the therapeutic effect of the medication. If patient has difficulty with slow deep breaths, an alternative is to keep mouth tight on mouthpiece and breathe slowly 2-3 times after depressing MDI. Ensure the physician's dosage instructions are followed and that only one puff of medication is delivered at a time.
6	Slow down inhalation if you hear the ***FLOWSIGnal™*** Whistle sound.
7	Follow instructions supplied with the MDI regarding the amount of time to wait before repeating instructions 3–5 as prescribed.

Trudell Medical International™
Manufactured by: Trudell Medical International,
725 Third Street, London, Ontario, Canada N5V 5G4
E-mail: **customerservice@trudellmed.com** Web site: **www.trudellmed.com**

Printed in Canada. P/N 106079-001 Rev B March 2005

AeroChamber MAX™
Valved Holding Chamber with Mouthpiece

For more information visit: **www.aerochambermax.com**
Replacement copies of this insert can be obtained by visiting www.trudellmed.com or by calling 1-866-510-0004
AeroChamber MAX® VHC Complies with CSA Standard Z264.1-02, Spacers and Holding Chambers for Use with Metered Dose Inhalers



Cleaning Instructions

	ENGLISH
	The ***AeroChamber MAX®*** VHC should be cleaned before first use and then weekly, following the instructions provided below.
1	Remove the Backpiece and the Mouthpiece Adapter Assembly as shown. Do not remove the Tethered Mouthpiece Cap from the Mouthpiece Adapter Assembly.
2	Soak all 3 parts for 15 minutes in a mild solution of liquid dish detergent and lukewarm clean water. Agitate gently.
3	Rinse parts in clean water.
4	Shake out excess water from the 3 parts and allow to air dry as shown. Ensure parts are dry before reassembly.
5	To reassemble, fit the Mouthpiece Adapter Assembly on the end of the chamber and twist firmly until components securely lock into position.
6	Center the Alignment Feature on the Backpiece with the Flow Indicator as shown. Press firmly to seat Backpiece. The protective Mouthpiece Cap should be placed on the mouthpiece when the product is not in use.

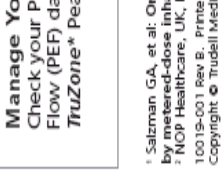
Cautions
- Ensure Directions for Use have been read prior to use and are kept available at all times.
- Do not leave ***AeroChamber MAX®*** VHC unattended with children.
- Do not disassemble the product beyond what is recommended in the Cleaning Instructions or damage may result.
- Review use of this device with your healthcare professional prior to use.
- The ***AeroChamber MAX®*** VHC is a medical device. To ensure proper performance it should only be cleaned according to these instructions. It should NOT be cleaned in a dishwasher.

Notes
- VHC = Valved Holding Chamber
- **This product contains no latex.**
- When not in use, store in suitable container.
- This product is designed to operate effectively for 24 months of continuous use providing the Directions for Use and Cleaning Instructions are followed and the device is not abused.
- **If you have questions about the performance or usability of this product, please contact your healthcare professional.**

AeroChamber MAX® VHC Product Selection Guide
- Infant Mask (0 - 18 months)
- Child Mask (12 months - 5 yrs.)
- Adult Mask or Mouthpiece (5 yrs. +)

THE LUNG ASSOCIATION
The Lung Association believes that the use of products such as ***AeroChamber MAX®*** VHC with your metered dose inhaler is helpful in delivering medication to the lungs.

Aerochamber MAX Patient Information Leaflet



Aerochamber PLUS package leaflet**:** patient instructions for use.

APPENDIX B ASTHMA TABLES

classifying severity in children who are not currently taking long-term control medication.

		Classification of Asthma Severity Children 0-4 Years of Age)			
			Persistent		
	Components of Severity	Intermittent	Mild	Moderate	Severe
Impairment	Symptoms	≤2 days/week	>2 days/ week but not daily	Daily	Throughout day
	Night-time awakenings	0	1-2x/month	3-4x/month	> lx/week
	SABA use for symptom control not prevention of EIB)	≤2 days/week	>2 days' week but not daily	Daily	Several times per day
	Interference with normal activity	None	Minor limitation	Some limitation	Extremely limited
Risk	Exacerbations requiring oral systemic corticosteroids	0-1 year	≥ 2 exacerbation in 6 months requiring oral corticosteroids or ≥ 4 wheezing episodes in 1 year lasting > 1day AND risk factors for persistent asthma		
		Consider severity and interval since last exacerbation. ← Frequency and severity may fluctuate over time. → Exacerbations of any severity may occur in any patients in any severity category.			

Level of severity is determined by both impairment and risk. Assess impairment domain by caregiver recall of previous 2-4 weeks. Assign severity to most severe category in which any feature occurs.
At present, there are inadequate data to correspond frequencies of exacerbations with different levels of asthma severity. For treatment purposes. patients who had ≥ 2 exacerbations requiring oral corticosteroids in the past 6 months, or ≥4 wheezing episodes in the past year. and who have risk factors for persistent asthma may be considered the same as patients who have persistent asthma, even in the absence of impairment levels consistent with Persistent asthma.
Classifying severity in patients after asthma becomes well controlled, by lowest level of treatment required to maintain control.

	Classification of Asthma Severity			
		Persistent		
	Intermittent	Moderate	Mild	Severe
EIB. exercise-induced bronchospam SABA. short-acting inhaled β_2-agonist	Step 1	Step 2	Step 3 or 4	Step 5

Classifying severity in children who are not currently taking long-term control medication.

		Classification of Asthma Severity Children 5-11 Years of Age)			
			Persistent		
	Components of Severity	Intermittent	Mild	Moderate	Severe
Impairment	Symptoms	≤2 days/week	>2 days/ week but not daily	Daily	Throughout day
	Night-time awakenings	≤2x/month	3-4x/month	>1 x/week but not nightly	Often 7x/week
	SABA use for symptom control not prevention of EIB)	≤2 days/week	>2 days' week but not daily	Daily	Several times per day
	Interference with normal activity	None	Minor limitation	Some limitation	Extremely limited
	Lung function	Normal FEV_1 Between exacerbations FEV_1 >80% predicted FEV_1/FVC >85%	FEV_1 >80% predicted FEV_1 /FVC >80%	FEV_1 = 60%-80% Predicted FEV_1 /FVC 75%-80%	FEV_1 <60% predicted FEV_1 /FVC <75%
Risk	Exacerbations requiring oral systemic corticosteroids	0-1 year	≥ 2 see note		
		Consider severity and interval since last exacerbation. Frequency and severity may fluctuate over time. Exacerbations of any severity may occur in any patients in any severity category. Relative annual risk of exacerbations may be related to FEV_1.			

Level of severity is determined by both impairment and risk. Assess impairment domain by patient/caregivers recall of the previous 2-4 weeks and spirometry. Assign severity to the most severe category in which any feature occurs.
At present, there are inadequate data to correspond frequencies of exacerbations with different levels of asthma severity. In general, more frequent and intense exacerbations e.g.. requiring urgent, unscheduled care, hospitalisation. or ICU admission indicate greater underlying disease severity. For treatment purposes. patients who had ≥2 exacerbations requiring oral systemic corticosteroids in the past year may be considered the same as patients who have persistent asthma, even in the absence of impairment levels consistent with persistent asthma.
Classifying severity in patients after asthma becomes well controlled, by lowest level of treatment required to maintain control.

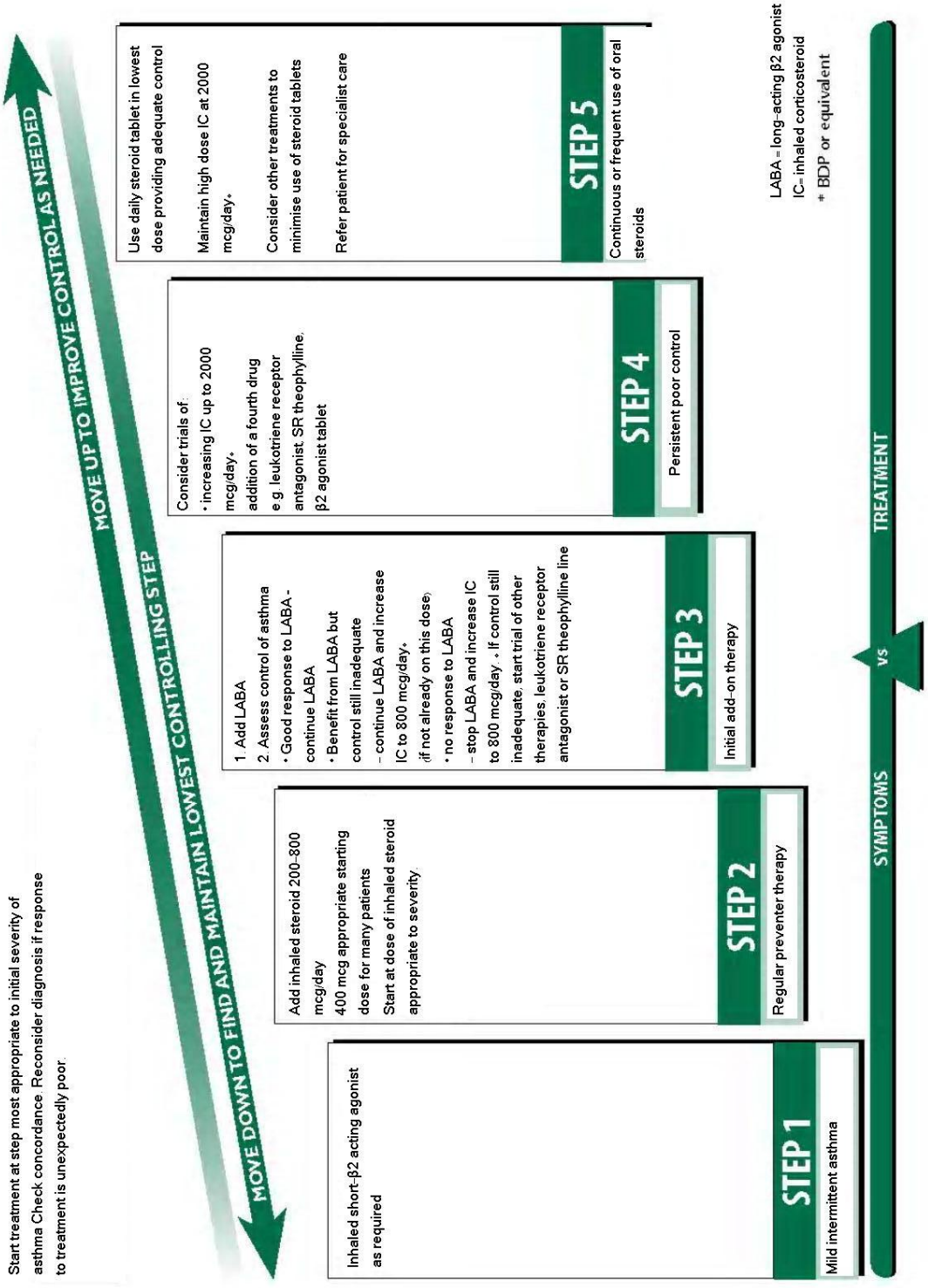
EIB. exercise-induced bronchospasin; FEV_1, forced expiratory volume in 1second: FVC. forced vital capacity ICU. intensive care unit. SABA. short-acting β_2 agonist.	Classification of Asthma Severity			
	Persistent			
	Intermittent	Moderate	Mild	Severe
	Step 1	Step 2	Step 3 or 4	Step 5

Classifying severity in children who are not currently taking long-term control medication.

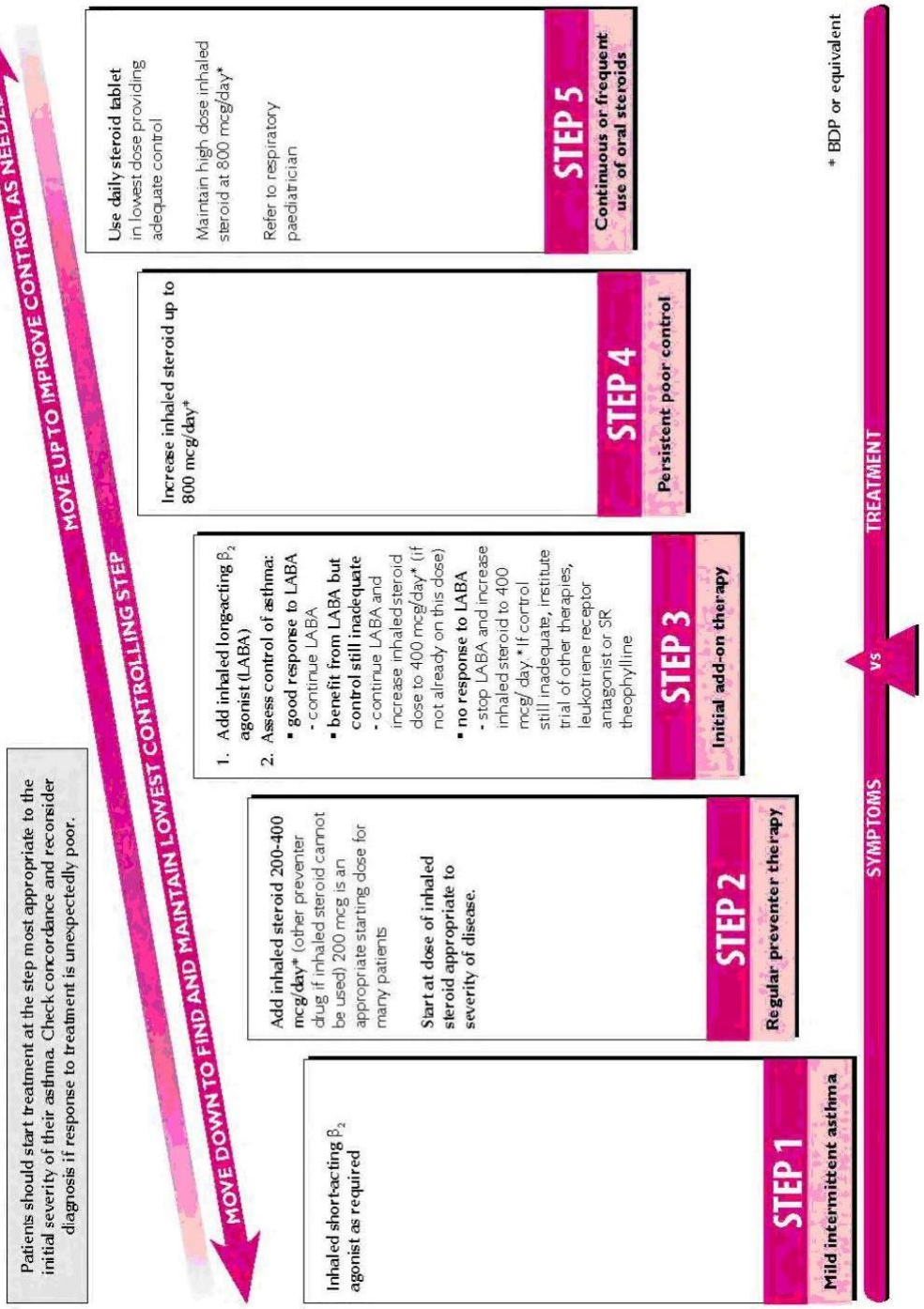
		Classification of Asthma Severity Children ≥ 12 Years of Age)			
			Persistent		
	Components of Severity	Intermittent	Mild	Moderate	Severe
Impairment	Symptoms	≤2 days/week	>2 days/ week but not daily	Daily	Throughout day
	Night-time awakenings	≤2x/month	3-4x/month	>1 x/week but not nightly	Often 7x/week
	SABA use for symptom control not prevention of EIB)	≤2 days/week	>2 days' week but not > 1x/day	Daily	Several times per day
	Interference with normal activity	None	Minor limitation	Some limitation	Extremely limited
	Lung function	Normal FEV_1 between exacerbations FEV_1 >80% predicted FEV_1/FVC normal	FEV_1 ≥80% predicted FEV_1 /FVC > normal	FEV_1 > 60% < 80% predicted FEV_1 /FVC reduced 5%	FEV_1 <60% predicted FEV_1 /FVC reduced > 5
Risk	Exacerbations requiring oral systemic corticosteroids	0-1 year	≥ 2 see note		
		Consider severity and interval since last exacerbation. Frequency and severity may fluctuate over time. Exacerbations of any severity may occur in any patients in any severity category. Relative annual risk of exacerbations may be related to FEV_1.			

Level of severity is determined by both impairment and risk. Assess impairment domain by patient/caregivers recall of the previous 2-4 weeks and spirometry. Assign severity to the most severe category in which any feature occurs.
At present, there are inadequate data to correspond frequencies of exacerbations with different levels of asthma severity. In general, more frequent and intense exacerbations e.g.. requiring urgent, unscheduled care, hospitalisation. or ICU admission indicate greater underlying disease severity. For treatment purposes. patients who had ≥2 exacerbations requiring oral systemic corticosteroids in the past year may be considered the same as patients who have persistent asthma, even in the absence of impairment levels consistent with persistent asthma.
Classifying severity in patients after asthma becomes well controlled, by lowest level of treatment required to maintain control.

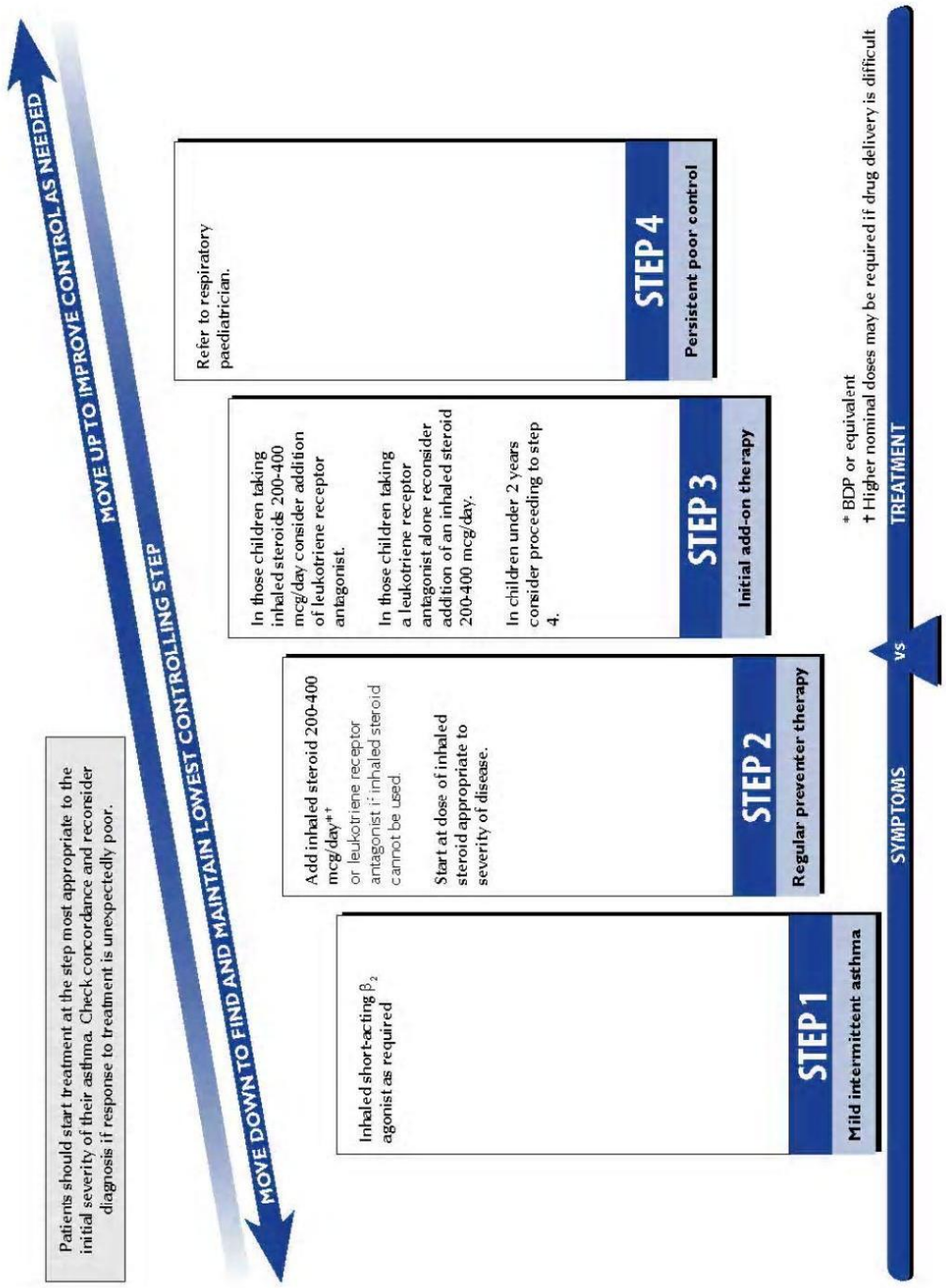
EIB. exercise-induced bronchospasin; FEV_1, forced expiratory volume in 1second: FVC. forced vital capacity ICU. intensive care unit. SABA. short-acting β_2 agonist.	Classification of Asthma Severity			
		Persistent		
	Intermittent	Moderate	Mild	Severe
	Step 1	Step 2	Step 3 or 4	Step 5



Summary of stepwise management in adults British Guideline on the Management of Asthma)



Summary of stepwise management in children aged 5-12 years British Guideline on the Management of Asthma)



Summary of stepwise management in children aged less than 5years British Guideline on the Management of Asthma)

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High performance liquid chromatography assay method for simultaneous quantitation of formoterol and the two epimers of budesonide

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Objective The aim of this study was to develop a sensitive and a simultaneous HPLC method for the analysis of formoterol and the two epimers of budesonide hence it is required by European pharmacopoeia to keep a fixed ratio between the two epimers.

Method This method used a Capital ODS2 Spherisorb 5 micrometer 250 mm 4.6 mm i.d. with a mobile phase consisting of acetonitrile phosphate buffer (pH 3.0; 7.5 mM) (40:60, v:v) and a flow rate of 1.0 mL/min; two wave lengths were used to analyse the pharmaceutical preparations, the first wave length was 214 nm for formoterol and the second was 240 nm for budesonide.

Result Validation studies demonstrated that the method possessed a linear UV response, high system precision and accuracy, high sensitivity and specificity for formatrol and two epimers of budesonide. The limit of detection (LOD) and the limit of quantitation (LOQ) for formoterol assay method were 5.401 mcg/L and 18.003 mcg/L respectively. In addition, the LOD and LOQ for budesonide A assay method were 134.525mcg/L and 448.416mcg/L, respectively. Finally, The LOD and LOQ for Budesonide B assay method were 62.27mcg/L and 207.566mcg/L, respectively.

Conclusion An isocratic liquid chromatographic method has been described, optimised and validated for simultaneous qualitative and quantitative determination of formoterol fumarate and budesonide epimers. Acceptable assay precision and accuracy and excellent linearity was achieved. In addition to its high sensitivity and robustness, the proposed HPLC method proved reliable determination of the budesonide and formoterol delivered from the Symbicort Turbuhaler. As result this method can substitute the two separate methods, and the single method for budesonide and formoterol since it can determine both budesonide epimers. For that reason, this method will save both cost and time.