

Full Length Research Paper

Aerodynamic characterization of marketed inhaler dosage forms: High performance liquid chromatography assay method for the determination of budesonide

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A sensitive and rapid high performance liquid chromatography method was developed and validated for the determination of aerodynamic characteristics of the emitted dose of budesonide from different inhaler dosage forms. The mobile phase consisted of a mixture of acetonitrile and 10 mM ammonium acetate (63:37% v/v) adjusted to pH5 with orthophosphoric acid. The HPLC analysis was performed at a flow rate of 1 mL/min using a C18 Zorbax Eclipse Plus column (250 x 4.6 mm, 5 μ) and an UV detection wavelength of 254 nm was used. The method was validated for specificity, linearity, precision, accuracy, limit of quantification, limit of detection, robustness and solution stability. The calibration curve was linear over a concentration range of 0.05 to 62.50 μ g/mL ($r^2 = 0.9999$) with limit of detection and limit of quantification of 0.02 and 0.06 μ g/mL, respectively. The intra-day and inter-day precision and accuracy were between 0.01 and 2.00% and -1.9 and 0.007%, respectively. The method was successfully applied to measure the amount of emitted and fine particle budesonide doses from Pulmicort Respules®, Pulmicort Inhaler® and Pulmicort Turbuhaler®.

Key words: Impactor, pulmicort, inhalation, HPLC, aerodynamic diameter, assay.

INTRODUCTION

Corticosteroids drugs are used in treatment of many conditions where these agents are central to reducing morbidity and mortality (D'Cruz, 2003; Sahib et al., 2009). Budesonide (BUD) is one of the corticosteroids representing the cornerstone of asthma management, enabling patients to enjoy a near normal or normal lifestyle (Gibson et al., 2001). This agent is available in different formulations, from metered dose (MDI) and dry powder inhalers (DPI) to products for nebulisation, to meet the needs of the heterogeneous population of asthmatic patients (Berger, 2009). Nevertheless, one of the major limitations of such inhalation products is the variability in the pulmonary drug deposition, which in turn leads to potential differences in clinical responses (Mitchell et al., 2007). In quality control analysis, time

and cost effective analysis are some of the important issues, which need to be addressed (Balaji et al., 2008; Bate et al., 2009; Rao and Nikalje, 2009; Sheshala et al., 2009).

It was therefore decided to explore the possibilities of developing a single analysis method of budesonide for different inhaler dosage forms, thus replacing the need for two or three separate methods and thereby saving the time and cost. A review of recent literature revealed that most HPLC methods published for BUD, involved the use of the internal standard (Peter and Chris, 1999; Vaghi et al., 2005; Assi et al., 2006) and high injection volume (Feddah et al., 2000). There is also insufficient information relating to the run time, selectivity and/or limit of detection (Bisgaard, 1998; Vaghi et al., 2005; Amani et al., 2010). In addition, other researchers reported limit of quantification of 0.1 μ g or more (Assi et al., 2006; Liljelind et al., 2007; Naikwade and Bajaj, 2008).

Therefore, the aims of the present study are to develop a simple yet sensitive HPLC method for the analysis of

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budesonide in different inhaler dosage forms and to use the developed method to compare the aerodynamic performances of three different budesonide inhaled products, namely Pulmicort Respules® (Budesonide 0.5 mg/mL; AstraZeneca), Pulmicort Inhaler® (Budesonide 0.2 mg/puff; AstraZeneca), and Pulmicort Turbuhaler® (budesonide 0.2 mg/puff; AstraZeneca).

EXPERIMENTAL

Method optimization

The present method used a slightly acidic mobile phase (pH 5) in order to prolong the lifespan of the column, since using a lower pH value might affect the material impact of the column, when used over a long period of time. Ammonium acetate was chosen as the buffer because it gives good peak shape with little interference and is easier to wash out from the column compared to phosphate buffer at the same pH value (Neue et al., 2005). In addition, this buffer is suitable for use in the future work, in order to determine the lung bioavailability of budesonide when using LC-MS-MS as its volatile buffer. During optimization of the present method, we tried to use methanol but it gave a high pressure and required a long period to run.

Apparatus and chromatographic conditions

The HPLC system consisted of a Shimadzu LC-20AD delivery pump (Shimadzu, Japan) equipped with the SIL- 20A HT prominence autosampler, (Shimadzu, Japan) fitted with 100 L sample loop, UV/Vis detector (SPD-20A, Shimadzu, Japan), DGU-20A3 prominence degasser (Shimadzu, Japan) and the chromatographic integrator (CBM-20A prominence communications bus model, Shimadzu, Japan). The chromatographic separation of the analyte was achieved at 40°C (CTO-10AS VP, Shimadzu column oven) using a zorbax eclipse plus (250 x 4.6 mm, 5 mm) analytical column. The mobile phase which was consisted of 10 mM ammonium acetate (pH 5 adjusted with orthophosphoric acid): acetonitrile (37:63) was filtered through a 0.45 µm nylon membrane filter (Whatman, UK) under vacuum and degassed prior to use. The analysis was carried out at a flow rate of 1.0 mL/min. The detector wavelength was set at 254 nm. The injection volume was 50 L. All solvents were of HPLC grade (J. T. Baker Analyzed, China). Budesonide was obtained from symbiotica specialty ingredients SDN. BHD (Kedah, Malaysia).

A stock solution of BUD was prepared by dissolving 50 mg of BUD in 50 mL of methanol in order to give the concentration of 1 mg/mL. The solution was protected from light by using an aluminum foil, as BUD suffers from a poor light stability (Gupta and Bhargava, 2006). Working solutions containing 0.05 to 62.5 µg/mL of BUD were prepared by serial dilutions of aliquots of the stock solution with the mobile phase. 50 L aliquots were injected (six times) and eluted with the mobile phase under the reported chromatographic conditions (Jenke, 1996). The average peak area versus the concentration of BUD in µg/mL was plotted and the corresponding regression equation was obtained.

RESULTS AND DISCUSSION

Method validation

The newly developed HPLC method was validated in

order to confirm that the present method was suitable for its intended purpose, as described in ICH guidelines Q2 (R1) (ICH, 2005). The above described method was validated in terms of linearity, specificity, precision, accuracy, limit of detection, limit of quantification, robustness, and solution stability.

Selectivity

The method was shown to be selective for BUD. Figure 1 shows a typical separation of budesonide (1 µg/mL). Analysis of mobile phase blanks confirmed that there were no interfering peaks due to the blank. In addition, the effect of inhaler excipients on the specificity of the developed HPLC method was also examined. The following excipients are present in the inhaler dosage forms: Pulmicort Inhaler® (magnesium stearate); Pulmicort Respules® (anhydrous citric acid, polysorbate 80, sodium chloride, disodium edetate, and sodium citrate); Pulmicort Turbuhaler® (no excipient). No significant interfering peaks from the excipients were found at the retention time of BUD (5.1 min). This showed that the developed analytical method was suitable for the analysis of BUD in different inhaler dosage forms. The chromatograms of BUD and excipients samples spiked with BUD at a concentration of 1 µg/mL are shown in Figure 1.

Linearity

To evaluate the linearity of the method, six calibration curves in the concentration range of 0.05 to 62.5 µg/mL (0.05, 0.1, 0.5, 2.5, 12.5, and 62.5 µg/mL) were prepared. The calibration curves were plotted for a peak area of the analyte against the corresponding concentration, which is obtained by using the linear regression analysis. The mean linear regression equation was, $y = 84.828 (\pm 0.02) x - 2.41 (\pm 0.5)$ with a correlation coefficient of 0.9999. The result showed that an excellent correlation existed between the peak area and concentration of the analyte. The result of linearity is presented in Table 1.

Intra-day and Inter-day precision and accuracy

Intra-day and inter-day precision and accuracy were evaluated by analyzing quality control samples at low, medium and high concentrations of 0.05, 2.50 and 62.50 µg/mL. For the intra-day variation, sets of six replicates were analyzed on the same day and for the inter-day validation, six replicates of three concentration levels were analyzed on three different days. The intra-day accuracy (relative standard error percent, % RE) ranged between -1.48 and 0.01% with a precision (relative standard deviation percent, % RSD) of 0.01 to 2.00%. The inter-day accuracy ranged between -0.01 and

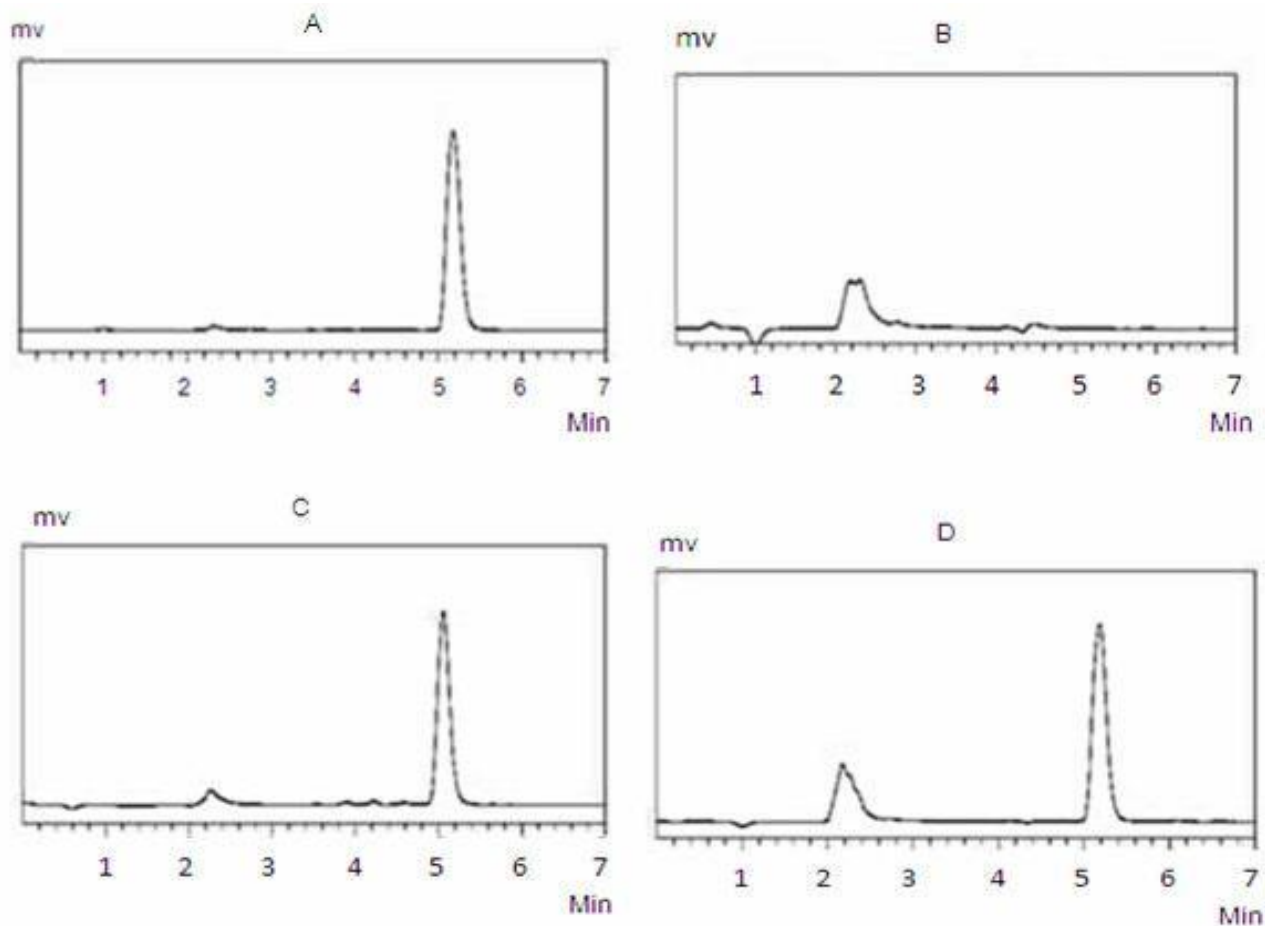


Figure 1. Typical HPLC chromatograms of budesonide (BUD). A: BUD, B: Placebo sample, C: excipients of Pulmicort Inhaler[®] spiked with BUD, D: Excipients of Pulmicort Respules[®] spiked with BUD. Retention time of BUD is 5.1 min.

Table 1. Summary of the calibration curve results for budesonide.

Theoretical amount (g/mL)	% of label claimed	% RSD	% RE
62.50	100.02 ± 0.01	0.01	0.02
12.50	99.50 ± 0.03	0.03	-0.50
2.50	100.14 ± 0.03	0.03	0.14
0.5	101.59 ± 0.97	0.95	1.59
0.10	99.08 ± 0.91	0.92	-0.92
0.05	99.23 ± 1.56	1.58	-0.77

Mean ± SD, N = 6.

-1.90% with a precision of 0.08 to 1.90%. All the results for precision and accuracy were within the acceptable limits (Epshtein, 2004). The results are shown in Table 2.

Limit of detection and limit of quantification

The sensitivity of the method was determined based on

the standard deviation of the response and the slope as described in ICH guidelines Q2 (R1) (ICH, 2005). The limit of detection (LOD) and quantification (LOQ) were calculated according to the following equations:

$$\text{LOD} = 3.3 / S; \text{LOQ} = 10 / S$$

where = the standard deviation of the response; S = the

Table 2. Experimental values of mean concentration, % RSD and % RE presented for validation parameters of budesonide.

Parameters	Theoretical budesonide concentration (g/mL)	% of label claimed	% RSD	% RE
Intra-day ^a	62.50	100.01 ± 0.01	0.01	0.01
	2.50	99.60 ± 0.52	0.52	-0.40
	0.05	98.51 ± 1.97	2.00	-1.48
Inter-day ^b	62.50	99.98 ± 0.09	0.09	-0.01
	2.50	99.50 ± 1.18	1.19	-0.50
	0.05	98.10 ± 1.87	1.90	-1.90
Short-term ^c	62.50	99.37 ± 0.01	0.01	-0.62
	2.50	100.10 ± 0.41	0.41	0.10
	0.05	99.31 ± 1.67	1.69	-0.68

^a Intra-day accuracy and precision was determined with 6 replicates for each concentration. ^b Inter-day accuracy and precision was determined with 6 replicates for 6 days for each concentration. ^c 14 days at 4°C, N = 6.

slope of the calibration curve.

The LOD and LOQ were found to be 0.02 g/mL and 0.06 g/mL, respectively.

Solution stability

Reference solutions were stored in the refrigerator for 14 days and re-analyzed in an injection sequence by employing freshly prepared standard solutions for a short-term stability. The above experiments were performed by using low, medium and high quality control samples. The drug was found to be stable in the above mentioned conditions. The solution stability results are shown in the Table 2.

Method robustness

The robustness of the method was assessed as a function of changing pH and changing acetonitrile and buffer volume ratio. The changes were in a range of ± 5% of the target (experimental condition). A resolution factor of greater than 2.0 min between all the peaks of the volume ratio was maintained to satisfy the system suitability criteria. The chromatographic response of the method indicated that the developed method was robust (the result is not shown here).

Application of the method: Aerodynamic characterization

Three different Pulmicort preparations were used in this part of the study. They are Pulmicort Inhaler® (MDI, this

is a breath-actuated inhaler which delivers the budesonide in a fine mist to the lung), Pulmicort Turbuhaler® (DPI, this is a dry powder inhaler which delivers the budesonide in a fine powder form to the lung) and Pulmicort Respules® (this is an inhalation suspension to be given by compressed air driven nebulizer).

The next generation impactor (NGI) (Copley, UK) was used to determine the particle size distribution of the pulmicort preparations. Upon impaction, the sample tested is divided into seven categories, which are characterized according to the aerodynamic diameter. The cutoffs, for the impactor at 60 L/min follow rate, are: 8.06 (Stage 1), 3.46 (Stage 2), 2.82 (Stage 3), 1.66 (Stage 4), 0.94 (Stage 5), 0.55 (Stage 6), and 0.34 (Stage 7). A vacuum pump was connected to NGI and operated at the flow rate of 60 L/min, which simulated the mean peak inspiratory flow rate (PIFR) of asthmatic adult patients (Engel et al., 1990). The flow rate was calibrated by using a flow meter (Copley, UK). The Pari LC Plus nebulizer (Germany) was loaded with 0.5 mg of Pulmicort Respules®, and compressed air from the Pari Master pump (Pari Master, Germany) was supplied to the nebulizer. Nebulization of the samples was carried out for 15 min at the room temperature (28°C) and a humidity of 65%. In contrast, Pulmicort Inhaler® and Pulmicort Turbuhaler® were primed with three sprays before use.

Samples were collected according to the following protocol: the vacuum pump was turned on; the Pulmicort Inhaler® sample was shaken for 5 s and then inserted into the mouthpiece adaptor on the induction port. The valve on the inhaler was then depressed for 1 s, expelling the spray; the pump was turned off after 5 s. Thirty seconds were then allowed to elapse before the second spray was collected in the same manner. This procedure was repeated for three times. An exception was Pulmicort Turbuhaler®, which was just inserted into the mouthpiece adaptor on the induction port and a single puff was

Table 3. Comparison of aerodynamic characteristic of different budesonide formulations.

Parameters	Pulmicort Respules®	Pulmicort Turbuhaler®	Pulmicort Inhaler®
MMAD ^a	4.48 ± 0.12	3.06 ± 0.03	3.38 ± 0.07
GSD ^b	2.00 ± 0.02	2.83 ± 0.12	2.25 ± 0.05
ED ^c	39.73 ± 0.52	52.94 ± 0.67	94.41 ± 0.35
FPF ^d	15.48 ± 0.61	28.44 ± 0.59	25.15 ± 1.18

Data represented as Mean ± SD, N = 3. ^a Mass median aerodynamic diameter. ^b Geometric standard deviation. ^c Emitted dose
^d Fine particle fraction size < 3.9 μm.

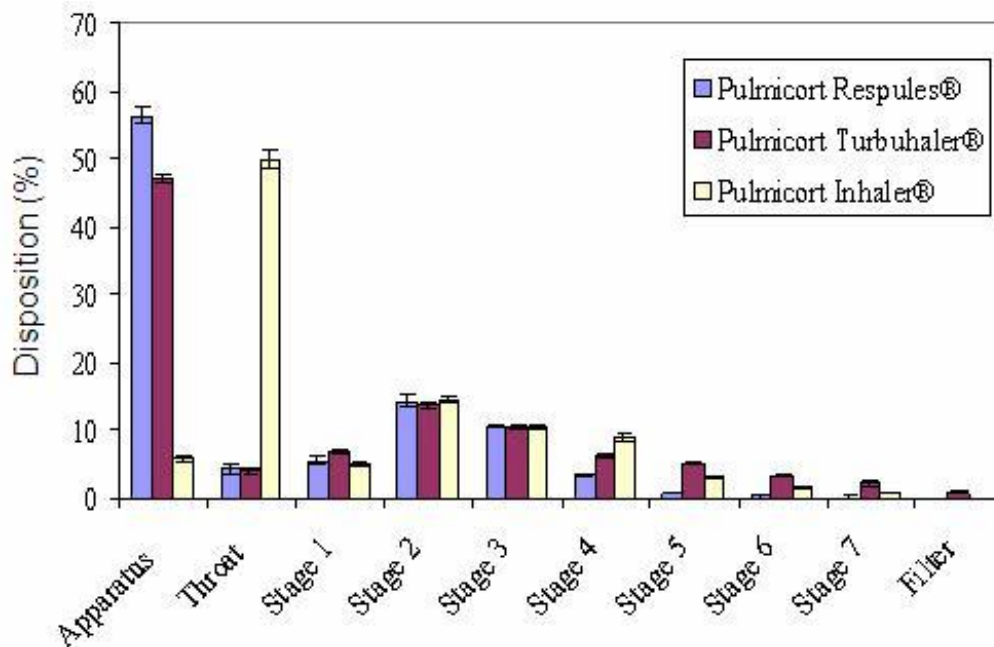


Figure 2. Distribution of aerosolized BUD formulations in the apparatus and NGI following nebulization at a flow rate of 60 ml/min. Mean ± SD, N = 3.

introduced into the impactor. The flow rate was allowed to continue for further 20 s. This was repeated for two extra puffs. As a consequence of the overall procedures, each stage of the apparatus, the induction port and pre-separator, and inhaler apparatus were rinsed with the mobile phase and collected in a 10 ml volumetric flask for quantitative analysis.

The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) (Table 3) were calculated after plotting the cumulative amount of drug deposited in each stage of the cascade impactor versus their corresponding aerodynamic diameter, as specified by the cascade impactor using a log-probability paper (Wiggins, 1991).

The result had shown a significant difference in MMAD and GSD for different formulations ($p < 0.05$). Figure 2 depicts the distribution of BUD from different formulations that remained in the apparatus, the induction port and

pre-separator (throat), and the different stages of the cascade impactor. These results were in agreement with previous *in vitro* reports, where the MMAD of the inhaler of budesonide was greater than the turbuhaler (Feddah et al., 2000). Since Pari LC Plus, a conventional jet nebulizer having a medium droplet size of 4 to 5 μm was used in this study, which might have caused a high MMAD of Pulmicort Respules®. In addition, there was a big difference in results obtained for the emitted doses for all preparations with the Pulmicort Respules® giving the lowest value. It has been reported that the amount of drug retained in the nebulizer was positively correlated to the relative droplet size produced by the nebulizers (Vaghi et al., 2005), and this might have contributed to the relatively larger amount of budesonide retained in the nebulizer and resulted in the lowest emitted dose. High concentrations of active ingredients (22 to 75%) being retained in the nebulizers were also reported (Darwis and

Kellaway, 2001; Vaghi et al., 2005).

The aerodynamic characteristics, from the perspective of the nebulization process, also depend on the type of nebulizer/compressor combinations (Berg and Picard, 2009). The deposition of the inhaled formulation in the induction port and pre-separator (which simulated the throat of the patient) ranked the highest for Pulmicort Inhaler®, followed by Pulmicort Respules® and Pulmicort turbuhaler®. The low amount of deposition of drug in the induction port and pre-separator suggested a reduction in the incidence of oropharyngeal fungal infection (Abdulla et al., 2010). Emitted dose (ED) and fine particle fraction (FPF) size < 3.9 µm were calculated as previously reported (Abdulla et al., 2010). The FPF in the order from the highest to lowest is: Pulmicort Turbuhaler® > Pulmicort Inhaler® > Pulmicort Respules®. In conclusion, caution should be taken, when switching from one inhaler dosage form to another as the actual dose delivered to the lungs is different and further *in vivo* studies may be warranted in light of the findings of the present research.

Conclusions

A new simple yet sensitive reverse phase liquid chromatography method was developed for the determination of budesonide in inhaler dosage forms. The validated method showed satisfactory results for all the validation parameters tested. The short retention time of around 5.1 min allowed the analysis of a large number of samples in a short period of time and it was therefore more cost effective. In addition, there was no interference from the formulation excipients. The developed method was successfully applied for the *in vitro* analysis of budesonide in commercially available inhaler dosage forms. The present method gave a reliable result as compared to the previously reported aerodynamic characteristic values, and thus it could be used for the quality control of budesonide inhaler dosage forms.

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