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Short communication

High performance liquid chromatography assay method for simultaneous quantitation of formoterol and budesonide in Symbicort Turbuhaler

K.H. Assi *, W. Tarsin, H. Chrystyn

Institute of Pharmaceutical Innovation, University of Bradford, Bradford BD7 1DP, UK

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Abstract

A sensitive and rapid high performance liquid chromatography method has been developed and used for the simultaneous determination of formoterol and budesonide in Symbicort Turbuhaler when assessing the aerodynamic characteristics of the emitted dose using Pharmacopoeial methods. This capability results in both time and cost saving.

The mobile phase composition was acetonitrile–5 mM sodium dihydrogen orthophosphate, pH 3 (60: 40% v/v), and was passed at 1.5 ml min^{-1} through a C_{18} column with a UV detection (wavelength 214 nm).

The method was shown to give good analytical performance in terms of linearity, precision (using phenylpropanolamine as an internal standard), sensitivity and solution stability. The intra-day precision for both formoterol and budesonide were 0.75% and 1.11%, respectively ($n = 10$). The limit of quantitation for formoterol was 10 μg L^{-1} and for budesonide was 120 μg L^{-1}, and the limit of detection were 3 and 30 μg L^{-1}, for both formoterol and budesonide, respectively.

The method has been applied to determine the content of the emitted dose and the fine particle dose of Symbicort Turbuhaler.

Keywords: Formoterol; Budesonide; HPLC; Assay

1. Introduction

Pharmacopoeial methods [1] describe the in vitro characterisation of the emitted dose from an inhaled product that is required by the Regulatory Authorities. These methods include the determination of the total emitted dose, the fine particle dose and the mass median aerodynamic diameter. Inhaled β-agonists and corticosteroids have been the cornerstone in the management of asthma and chronic obstructive pulmonary disease (COPD). Recently inhaled combinations of a long acting and a corticosteroid have been recommended in the management of asthma [2] and COPD [3]. Inhalation of the two drugs as one dose in combination inhalers has been shown to be more clinically effective [4]. This has lead to the introduction of combination inhalers. A combination of formoterol and budesonide is now available for inhalation from a Turbuhaler inhaler (Symbicort-Draco Läkemedel AB, Sweden). Symbicort has been labelled to deliver 6 μg (4.5 μg emitted) of formoterol with 100 μg (80 μg emitted) and 200 μg (160 μg emitted) of budesonide in the same dose and has been shown to be more effective than budesonide in the management of asthma [5] and COPD [6].

There is an increasing drive towards achieving time and cost effective analysis. It was therefore decided to explorer the possibilities of developing a simultaneous analysis of formoterol and budesonide, so replacing the need for two separate methods and thereby saving time and cost. The purpose of this work was thus to develop a sensitive HPLC method for the analysis of formoterol and budesonide involved in the in vitro Pharmacopoeial methods for the dose emitted from inhalers.

2. Experimental

The HPLC system was a Gilson model 302 pump and a Gilson model 231 autosampler equipped with a 200 μL volume loop. Chromatographic separation was performed using a 250 mm × 4.6 mm i.d. (5 μm particle size) Spherisorb C_{18} column (Waters, UK) Chromatographic data were collected and

* Corresponding author.
E-mail address: khaassi@bradford.ac.uk (K.H. Assi).

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analysed on a Shimadzu Chromatopac CR-6A data processor (Tokyo, Japan). The detector wavelength was set at 214 nm.

The mobile phase was acetonitrile–5 mM sodium dihydrogen orthophosphate, pH 3 (60:40%, v/v). The mobile phase was filtered under vacuum through a 0.45 μm filter (Gelman Science, Germany) and degassed in an ultrasonic bath under vacuum for 10 min. Budesonide, phenyl propanolamine (internal standard) and formoterol were injected into the system and separated at 30 °C, using a constant flow rate of 1.5 ml min^{-1}.

All solvents were HPLC grade (BDH Chemicals Ltd., Poole, England). Sample solutions were prepared in the mobile phase. Formoterol was obtained from ML Laboratories PLC, phenylpropanolamine and budesonide were purchased from Sigma Chemical.

3. Result and discussion

3.1. Method optimisation

Two previous methods for the determination of formoterol [7] and budesonide [8] were adapted and optimised to allow one single method for routine long-term operation. Sixty percent acetonitrile and 1.5 ml min^{-1} flow rate were found to give fast separation time with high resolution between the separated peaks, methanol was tried but it gave a longer run time with higher column pressures due to its high viscosity when mixed with water. The two wavelengths 214 nm [6] and 250 nm [7] were tested, the 214 nm gave a three-fold increase in sensitivity for budesonide.

The presence of residual silanol groups on the silica surface caused peak tailing problems and an extended run time when operating at pH 7. In the literatures a number of approaches have been adopted to overcome this problem such as ion suppression [9], ion pair chromatography [10] or by the addition of a deactivating agent such as an aliphatic amine to the eluent [11,12]. In this study the pH of the mobile phase was lowered to pH 3 to avoid these problems. When the pH is lowered, the silanols become protonated [13] and thus eliminating the attractions between the ionised silanol groups and the NH_2 groups of the solutes.

3.2. Method validation

3.2.1. Selectivity

The method was shown to be selective for formoterol and budesonide. Fig. 1 shows a typical separation of a test mixture of formoterol (200 μg L^{-1}) and budesonide (1000 μg L^{-1}) with phenylpropanolamine (500 μg L^{-1}) as the internal standard, all dissolved in the mobile phase. Analysis of mobile phase blanks confirmed that there were no interfering peaks due to the blank.

3.2.2. Linearity

The detector response was shown to be linear over the range of 50–400 μg L^{-1} and 250–2000 μg L^{-1} for both formoterol and budesonide, respectively. The calibration solutions were diluted with internal standard solution (500 μg L^{-1}, phenylpropanolamine). Each solution was injected in duplicated

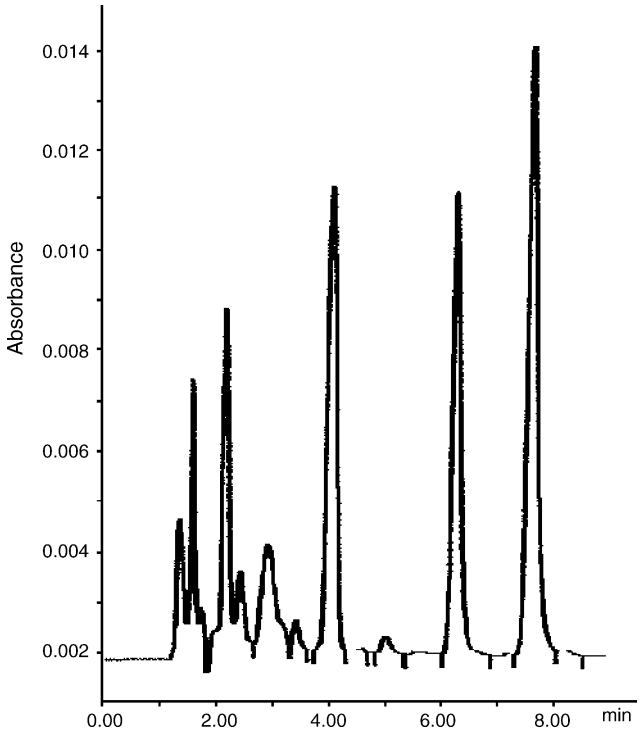

Fig. 1. Chromatogram of formoterol (200 μg L^{-1}), budesonide (1000 μg L^{-1}) and the phenylpropanolamine (500 μg L^{-1}) internal standard. Peak identities: budesonide 4.16 min, phenylpropanolamine 6.33 min and formoterol 7.72 min.

together with a blank of the internal standard solution. The linear response for formoterol gave a correlation coefficient of 0.994 ($y = 0.0041x + 0.0021$; $n = 5$) and a correlation coefficient of 0.992 ($y = 0.0006x + 0.009$; $n = 5$) was obtained for budesonide.

3.2.3. Limits

The limit of detection (signal-to-noise ratio = 3:1) for formoterol was 3 μg L^{-1} and for budesonide was 30 μg L^{-1}, the limit of quantitation (signal-to-noise ratio = 10:1) for formoterol was 10 μg L^{-1} and for budesonide was 120 μg L^{-1}. Three samples of both formoterol and budesonide were prepared at the quantitation limits and were analysed ($n = 10$), the relative standard deviation (R.S.D.) for both formoterol and budesonide were 3.81% and 4.65%, respectively.

The sensitivity of the method is not an issue in this application as the linearity range was selected to cover the concentration of both formoterol and budesonide in the emitted dose and the fine particle dose of Symbicort Turbuhaler. However, the LOQ was calculated to show that the method could be applied for lower concentration of analytes.

3.2.4. Precision

A standard solution of a 200 μg L^{-1} formoterol and 1000 μg L^{-1} budesonide containing the phenylpropanolamine internal standard of 500 μg L^{-1}, was used to test system precision. The intra- and inter-day variations were determined by calculating the relative standard deviation. The intra-day variations for both formoterol and budesonide were 0.75% and 1.11%, respectively ($n = 10$).

Table 1
Inter-day precision for both formoterol and budesonide

Days	Budesonide		Formoterol	
	Mean (peak area ratio)	S.D.	Mean (peak area ratio)	S.D.
1	1.119	0.013	0.433	0.002
2	1.176	0.012	0.454	0.003
3	1.197	0.003	0.460	0.002
4	1.155	0.009	0.448	0.005
5	1.203	0.013	0.460	0.003
Mean	1.170		0.451	
R.S.D.	2.920		2.537	

The inter-day variation was calculated by analysing the same solution for next 5 days ($n = 10$ each day), the R.S.D. for both formoterol and budesonide were 2.5% and 2.9%, respectively (Table 1).

3.2.5. Solution stability

Reference solutions were stored in the refrigerator for 14 days and re-analysed in an injection sequence employing freshly prepared standard solutions. No significant differences were found following storage. The concentrations after such storage conditions were 99% for formoterol and 98% for budesonide of the concentration values found with the freshly prepared solutions. Longer storage periods may be possible but were not assessed in this study.

3.2.6. Method robustness

The method robustness was assessed as a function of changing the pH and changing the acetonitrile and buffer concentration, the changes were over a range of ±5% of the target (experimental condition). The method system suitability criteria of a resolution grater than 2.0 between all peaks were maintained.

3.2.7. Accuracy

The accuracy of the method was performed by adding the analyte in blank matrices. Three different concentrations (low, medium and high) of the linear range 50–400 $\mu g\,L^{-1}$ and 250–2500 $\mu g\,L^{-1}$ for both formoterol and budesonide, respectively, were used ($n = 5$ for each level). Table 2 shows the percent recovery.

Table 3
Percentage of the nominal dose of formoterol and budesonide emitted from Symbicort Turbohaler at a pressure drop of 4 kPa across the inhaler

Dose no.	Formoterol	Budesonide
2	78.9	66.7
3	76.2	59.4
4	75.1	76.4
59	77.5	80.1
60	82.1	91.3
61	89.5	76.5
62	84.0	102.4
118	75.1	111.5
119	65.8	76.3
120	75.2	55.3
Mean	77.9	79.6
S.D.	6.4	17.9
R.S.D.	8.2	22.4

4. Application of the method

The pharmaceutical performance of inhaled products can be characterised by the total emitted dose, the fine particle dose and the aerodynamic particle size distribution. This HPLC method was used to assay the content of the emitted dose and the fine particle dose of Symbicort Turbuhaler.

4.1. Dose content uniformity

The method was useful to simultaneously analyse formoterol and budesonide in the emitted dose of Symbicort (80/4.5) Turbuhaler. The emitted dose uniformity was measured using a dose sampling apparatus described in the United State Pharmacopaeia 2005 [1]. Ten individual doses (dose number 2, 3, 4, 59, 60, 61, 62, 118, 119 and 120) of the entire dose available (120 doses) were collected from the Symbicort at a pressure drop of 4 kPa across the inhaler. The flow duration was 4.1 s, this was to allow a volume of 4 L to be drawn through the inhaler.

Each dose was collected and then was transferred to a 50 ml volumetric flask. It was diluted up to volume with internal standard solution (500 $\mu g\,L^{-1}$, phenylpropanolamine), to give concentrations of 120 and 2000 μg/L for both formoterol and budesonide, respectively.

The HPLC data was then compared with the nominal dose of Symbicort inhaler (Table 3). The R.S.D. value is high because of the high inter-dose emission variability from a Symbicort inhaler [14].

Table 2
Accuracy data for formoterol and budesonide contents

Budesonide			Formoterol		
Concentrations levels (μg/L)	Recovery (%)		Concentrations levels (μg/L)	Recovery (%)	
	Mean	R.S.D.		Mean	R.S.D.
Low = 500	97.23	0.70	Low = 100	98.56	0.43
Medium = 1000	96.95	0.95	Medium = 200	98.57	0.50
High = 2000	96.64	0.96	High = 400	99.00	0.94

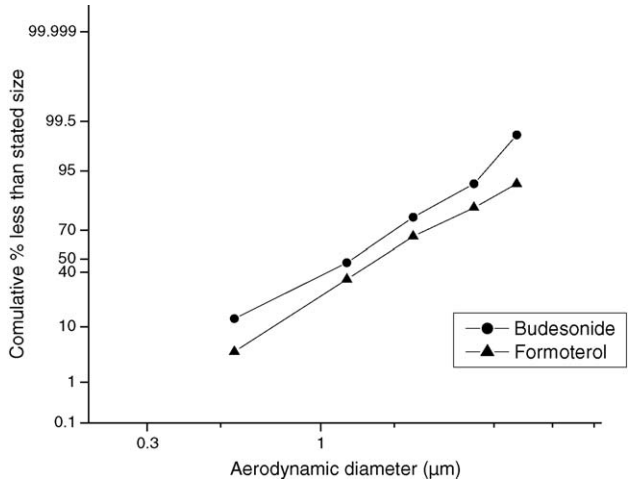

Fig. 2. Cumulative frequency (probability scale) against the log of aerodynamic diameter for Symbicort Turbohaler using Andersen MKII Cascade Impactor.

4.2. Particle size distribution

The particle size distribution and the fine particle mass from the Symbicort (80/4.5) Turbuhaler (budesonide/formoterol) were measured using the Andersen MKII Cascade Impactor, the flow rate through the mouthpiece was set at a pressure drop of 4 kPa across the inhaler. Ten consecutive doses were discharged into the Andersen MKII Cascade Impactor and for each dose the pump was switched on for 4.1 s (equivalent to an inhaled volume of 4 L drawn through the inhaler) with the inhaler in situ ready to deliver each dose. The fine particle size for formoterol and budesonide was 1.71 and 37.8 μg, respectively. The probability of the cumulative percentage of mass less than a stated particle size was plotted against the log of aerodynamic diameter (μm) as shown in Fig. 2. The mass median aerodynamic diameters (MMAD) were 1.5 and 1.2 and the geometric standard deviation (G.S.D.) were 1.8 and 1.9 for both formoterol and budesonide, respectively.

5. Conclusion

A HPLC method has been optimised and validated for the simultaneous determination of budesonide and formoterol in aerosol formulation. The method, which gave precise and accurate results, can substitute the two separate methods, which are currently used to determine the budesonide and formoterol delivered from the Symbicort Turbuhaler. Therefore, this method will save both cost and time.

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