Appendix

Methods

Drug identification

Drug identification was performed by elution, which is the use of a solvent mixture that promotes the separation of its components. The identification was performed by comparing the elution time of the ingredients through an HPLC system column (Prominence; Shimadzu Corp., Kyoto, Japan) with the elution time of the analytical standards. For the analysis, we used 10 capsules of the fixed-dose combination of formoterol/budesonide 12/400 µg of the test formulation, as well as 10 capsules of formoterol 12 µg and 10 capsules of budesonide 400 µg of the reference formulation.

Average capsule content weight

To determine the average capsule content weight, we used 20 capsules of the fixed-dose combination of the test formulation and 40 capsules of the reference formulation (20 capsules of each active ingredient). The capsules were opened, and the powder was removed and weighed with a precision analytical scale (AT 200; Mettler Toledo, Greifensee, Switzerland).

Active content (dosing)

The active content (dosing) was determined by HPLC (Shimadzu Corp.) with UV detection. The steps were as follows:

- extraction of the actives in the product by using an appropriate solvent
- injection of the active ingredient dissolved in the solvent into an HPLC separation column
- passage of an appropriate mobile phase through the separation column
- separation of the injected sample components on the basis of their different affinities towards the column
- elution of the sample components in the column, with identification and dosing of these components with a spectrophotometric detector, which determines the absorbance of the components at a chosen wavelength
- comparison of retention times between the standard and the samples

For formoterol, the standard retention time is approximately 4.6 min. For budesonide, the isomers exhibit two signals, with retention times of approximately 18.3 and 19.3 min.

Content uniformity

Content uniformity determination, the aim of which is to investigate variability in the concentrations of active ingredients in a pharmaceutical formulation, was performed in 10 capsules of the test formulation and in 20 capsules of the reference formulation (10 capsules of each active ingredient). The capsules were processed individually. The extraction liquid was analyzed by HPLC-UV, as described above for dosing determination.[7]

Delivered dose uniformity

The test for delivered dose uniformity is required for inhaled medications containing the active ingredient either in reservoirs or in premetered dosage units, and for active ingredient formulations packaged in reservoirs or in premetered dosage units where these containers are labeled for use with a named inhalation device. The target-delivered dose is the expected mean active ingredient content for a large number of delivered doses collected from many inhalers of the chosen product. In many cases, its value may depend upon the manner in which the test for delivered dose is performed.[6]

For determination of delivered dose uniformity, 10 containers and 10 inhalers were evaluated for the test and reference formulations. To that end, a dosage unit sampling apparatus for dry

powder inhalers (DUSA-DPIs; Westech Scientific Instruments, Bedfordshire, UK; Figure 1) was used (including base plate, large clamp, stand, and boss head clamp).[6] Thirty analyses were performed for the test formulation, and 60 analyses were performed for the reference formulation.

The following mode of procedure was settled upon for the test formulation:

- Beginning: first capsule in the container (first use of the inhaler), emptying and dispensing of the intermediate capsules (2nd to 14th capsule)
- Middle: 15th capsule (any capsule in the container), emptying and dispensation of the intermediate capsules (16th to 29th capsule)
- End: 30th capsule (any capsule in the container)

The following mode of procedure was settled upon for the reference formulation:

- Beginning: first capsule in the container (first use of the inhaler), emptying and dispensing of the intermediate capsules (2nd to 29th capsule)
- Middle: 30th capsule (any capsule in the container), emptying and dispensation of the intermediate capsules (31st to 59th capsule)
- End: 60th capsule (any capsule in the container)

Aerodynamic particle size distribution

An effective system for measuring aerodynamic particle size distribution is the Andersen cascade impactor (ACI).[8] The ingredient content in the discharged spray from the inhaler is drawn, by vacuum at a controlled flow rate, through a set of filters that mimic in vitro the airways up to the pulmonary alveoli (Figure 2).

We used the ACI model 8301-60 (Copley Scientific Ltd., Nottingham, UK), which separates the sample into fractions on the basis of differences in inertia, which is a function of particle size, shape, and velocity. The device includes a number of stages (filters), with a different number of perforations of known diameter, which decreases with increasing stage number. At each stage, particles with insufficient inertia to follow the air stream collect on the filter located beneath the stage, whereas the remaining particles pass onto the next stage. These particles are pulled by the air stream, at a constant flow rate produced by a vacuum pump, which means that smaller particles achieve sufficient inertia to collect on the finer filters.

Operational details for obtaining results

The system used received its qualification, which is valid for two years, on June 9, 2006. The plates/filters were coated with a 1% solution of silicone oil in hexane. The mouthpiece is made of silicone, in a manner consistent with the inhalers of the test and reference formulations analyzed. The plate and filters are positioned in a standard fashion, according to the model of the device.

The entire system was calibrated with empty capsules of each formulation. The inhalation volume was corrected using the following equation:

$$ECD' = SCD \times (28.3/Q)\hat{}0.5 \qquad (1)$$

where Q is the flow rate in L/min used in the analysis, ECD' is the effective cut-off diameter used in the analysis, and SCD is the standard cut-off diameter for a flow rate of 28.3 L/min.

The value of Q used in the above equation for the test and reference formulations was 94 L/min and 99 L/min, respectively.

The fine particle fractions (FPFs) and the fine particle doses (FPDs) were calculated on the basis of the mass of active ingredient found on each stage per delivered dose and after correction of the cut-off filter diameters as a function of the flow rate Q used in the analyses. This standard flow rate was adjusted to 90 L/min. The correction of the filter diameter for each ACI stage is dependent on the flow rate used in the tests. The values for the FPD and the FPF were calculated by the following formulas:

$$FPD = \sum m \div n \qquad (2)$$

where ∑m is the total mass of active ingredient found on stages 1 to 7 , and n is the number of sprays discharged (n = 10 in the present study).

$$FPF = (FPD \div DDU) \times 100 \quad (3)$$

where DDU is delivered dose uniformity in µg.

Calculations were performed with the Copley Inhaler Testing Data Analysis Software (CITDAS), version 3.10 (Copley Scientific Ltd.).

Water content

A Karl Fischer system (787 KF Titrino; Metrohm AG, Herisau, Switzerland) and a scale (AEU Libror 210; Shimadzu Corp.) were used for determining the water content. We analyzed five capsules of the test formulation and five capsules of each active ingredient of the reference formulation.

Volume variability

To evaluate the test formulation with low inspiratory volumes (simulating a patient with breathlessness), we performed testing of aerodynamic particle size distribution with a multistage liquid impinger (Astra Draco MSLI; Erweka, Heusenstamm, Germany), which assesses in vitro the effect of inhalation on particle size distribution. We used test formulation (formoterol/budesonide 12/400 µg) capsules from batch 1005968, which was manufactured in September of 2010 and was good through March of 2012. The testing was performed between October 20 and November 17, 2010.

The MSLI system makes it possible to measure aerosol particle size. This system has four impaction stages, each containing a small amount of liquid that is used to minimize particle re-entrainment (from an upper to a lower stage). An after filter is placed below the final stage. The theoretically expected cut-off diameters (in µm) when the flow rate is 60 L/min on the four stages and on the filter are, respectively, 13.0, 6.8, 3.1, 1.7, and < 1.7 µm.

The sample was obtained from five actuations of the flow control device, with two inspiratory volumes, and from recovery of material in the MSLI system by using an appropriate diluent and flow rate. These volumes were sufficient to ensure complete emptying of the capsule, as recommended in the directions for use. The times required to generate inspiratory volumes of 1 L and 4 L were previously calculated (0.7 s and 2.7 s, respectively).