Available online at www.sciencedirect.com
SCIENCE DIRECT
International Journal of Pharmaceutics 313 (2006) 14–22
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Co-deposition of salmeterol and fluticasone propionate by a combination inhaler☆

Andrew Theophilus[a], Alison Moore[b], Dave Prime[c], Simona Rossomanno[d], Brandon Whitcher[e], Henry Chrystyn[f,*]

[a] *Department of World-wide Physical Properties, GSK R&D, Ware, UK*
[b] *Clinical Development and Medical Affairs, GSK R&D, Greenford, UK*
[c] *Inhalation Product Development, GSK R&D, Ware, UK*
[d] *Department of Pre-clinical Development Statistics, GSK R&D, Ware, UK*
[e] *Research Statistics Unit, GSK R&D, Harlow, UK*
[f] *Institute of Pharmaceutical Innovation and School of Pharmacy, University of Bradford, Richmond Road, Bradford BD7 1DP, UK*

Received 19 July 2005; accepted 4 January 2006
Available online 3 March 2006

Abstract

The combination of the long-acting β2-agonist, salmeterol xinafoate (salmeterol) and inhaled corticosteroid, fluticasone propionate (FP) (Seretide®/Advair®) has shown enhanced efficacy compared with concurrent administration of the two drugs from individual inhalers at the same dose. A possible explanation for this increased effect is a higher degree of co-deposition of the two drugs from the combination (Seretide) inhaler compared with the component drugs administered separately.

Raman laser spectroscopy, a technique capable of identifying individual drug particles, has been used with novel statistical methodology that we have developed, to determine whether there is any co-association between drug particles and whether this occurs in the Seretide formulation rather than by chance. Samples from a combined Seretide metered dose inhaler (MDI, 25/50 mcg) and salmeterol (25 mcg) with FP (50 mcg) from separate MDI's taken from Plate 4 of an Anderson Cascade Impactor were analysed.

Using a statistical test based on the bootstrap technique, it was found that the co-deposition of FP and salmeterol particles from the combination MDI was significantly greater than from the separate inhalers group ($p < 0.001$). A higher degree of co-deposition on the same cells of the airways may possibly account for the increased efficacy observed in patients prescribed Seretide MDI.

Keywords: Synergy; Salmeterol; Fluticasone propionate; Combination therapy

1. Introduction

Asthma is a two-component disease characterised by airway inflammation and smooth muscle dysfunction (Johnson, 2002). The most effective treatment therefore, is one which targets both components of the disease. It has been demonstrated that addition of a long-acting β2-agonist (LABA) to an inhaled corticosteroid (ICS) is superior to ICS alone in achieving asthma control (Greening et al., 1994; Woolcock et al., 1996; Shrewsbury et al., 2000; Matz et al., 2001) resulting in this treatment approach being recommended in asthma guidelines (NHLBI, 1997; GINA, 2003; BTS, 2004). Inhalers combining a LABA and an ICS are now available; one such combination is salmeterol xinafoate (hereafter referred to as salmeterol) and fluticasone propionate—Seretide®/Advair® inhaler (GlaxoSmithKline, UK) and the other is a combination of formoterol with budesonide in Symbicort® (AstraZeneca, UK). Data have shown that when salmeterol and fluticasone propionate are combined, in the Seretide Diskus, there is an enhanced clinical benefit when compared to the individual drug components administered separately (Nelson et al., 2003). These differences have been demonstrated in controlled, double-blind, double-dummy studies in which increased compliance from the single inhaler over two concurrent inhalers could not be the reason for

☆ Seretide®, Advair®, Diskus®, Serevent®, Flixotide® are registered trademarks of the GlaxoSmithKline group of companies. Symbicort® is a registered trademark of AstraZeneca.

* Corresponding author. Tel.: +44 1274 233495; fax: +44 1274 236490.
E-mail address: H.Chrystyn@bradford.ac.uk (H. Chrystyn).

0378-5173/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2006.01.018

the improved effect (Aubier et al., 1999; Bateman et al., 1998; Chapman et al., 1999; Van den Berg et al., 2000). This increased efficacy observed in the clinic between the combination of salmeterol and fluticasone propionate, may not be a class effect as it has not been observed with the other LABA/ICS combination, budesonide/formoterol in Symbicort (Zetterström et al., 2001). The improvement in morning peak expiratory flow seen with Symbicort over the two drugs administered separately was not sustained over the course of the 12-week study.

An explanation for the clinical effect seen with Seretide could be due to a synergistic interaction at the receptor, molecular and cellular level between salmeterol and fluticasone propionate (Barnes, 2002). For this effect to occur, both drugs need to be present on the same cell in the airways. Administration of the two drugs via a single inhaler would lead to an increased likelihood of co-deposition compared with administration via two separate inhalers. This is because natural variation in inspiratory manoeuvres, causing different deposition patterns within the lung between inhalations from two separate inhalers, is avoided when both drugs are delivered in a single breath. It is possible that the opportunity for synergistic action is further enhanced in the Seretide formulation by co-deposition of fluticasone propionate and salmeterol particles in the airways as a result of particle co-association within the device.

We have therefore undertaken a programme of in vitro analyses to determine whether co-association of salmeterol and fluticasone propionate within the Seretide formulation could explain this synergistic effect. Raman laser spectroscopy has been applied to particles deposited on the stages of an Andersen Cascade Impactor. This method is capable of identifying individual drug particles and presents images where particles are colour coded. Previously this technique has been shown to highlight deposition of beclometasone and salbutamol from a Ventide metered dose inhaler (Fraser Steele et al., 2004). The ratios of salbutamol: beclomethasone identified by Raman Spectroscopy were similar to that of the quantified amounts but an assessment of co-deposition was not made. This Raman technique has been used to analyse Seretide and concurrent samples of salmeterol and fluticasone propionate taken from Plate 4 of an Anderson Cascade Impactor, the plate considered to represent the dose delivered to the central airways (Andersen, 1985). We elected to use the MDI device to simplify analysis of the Raman data since only salmeterol and fluticasone propionate particles are present. Numerous clinical studies have shown that the Seretide MDI and Diskus are equivalent (Bateman et al., 2001; van Noord et al., 2001; Emeryk et al., 2003). We have used a novel adaptation of statistical methodology to determine whether the likelihood of any observed co-deposition between the drugs happens by co-association in the formulation rather than by chance.

2. Materials and methods

2.1. Drugs evaluated

A metered dose inhaler (MDI) containing the COMBINATION of 25 mcg salmeterol with 50 mcg fluticasone propionate per actuation (Seretide, GlaxoSmithKline, UK) and SEPARATE INHALERS containing salmeterol (25 mcg, Serevent®, GlaxoSmithKline, UK) and fluticasone propionate (50 mcg, Flixotide®, GlaxoSmithKline, UK) per actuation were used in this analysis.

2.2. Sample and cascade impactor plate generation

A standard Anderson Cascade Impactor was set up in the normal configuration for analysis of MDI products (BP2002). The pump was set running at 28.3 l/min. In the case of Seretide (COMBINATION), a single actuation of the 25/50 mcg inhaler was fired into the impactor. For the drugs given separately (SEPARATE INHALERS), a single actuation of salmeterol 25 mcg was fired into the impactor followed by a single actuation of 50 mcg of fluticasone propionate. The stack was then dismantled and Plate 4, representing particles in the size range between 2.1 and 3.3 μm, which is generally thought to be representative of the central airways (Andersen, 1985), removed for Raman analysis. This procedure was repeated four times for the COMBINATION (sample numbers ra0579, ra0580, ra0582 and ra0584) and for SEPARATE INHALERS (ra0577, ra0581, ra0583 and ra0585). Thus eight stage 4 plates were analysed by Raman spectroscopy.

2.3. Raman equipment setup

Raman spectroscopy was performed using a LabRam Infinity Raman microscope (JY-Horiba) equipped with a 785 nm diode laser rated at 500 mW and a peltier cooled charged couple device (CCD) detector. A 100× objective lens was used on the microscope. The confocal hole was set at 65 μm and a grating with 600 groves/mm was used. The grating was centred on 1250 cm^{-1} and data collected over the spectral range 190–2140 cm^{-1}. Using this configuration the spatial resolution was estimated as approximately 3 μm and the depth of field resolution estimated as approximately 3.2 μm.

Control of the instrument, data capture and data evaluation were effected by a PC running LabSpec Software 4.02 (JY-Horiba). Although the resolution of the Raman microscope is approximately the same as the sizes of the individual particles under investigation this resolution provides the ability to clearly distinguish between particles of a single drug and particles of a combination.

2.4. Imaging process

Plate 4 from the Cascade Impactor was placed on the Raman microscope stage with a single deposit of impacted particles located below the objective lens (Fig. 1). The position of the plate was adjusted visually using the optical microscope to obtain a field of evenly spaced particles. This optical micrograph, approximately 175 μm × 250 μm, was captured electronically for future reference. The system was switched from optical to spectroscopy mode and a spectrum of the centre of the image obtained to check correct instrument setup.

Imaging was achieved using a XY motorised stage. An area of 128 μm × 128 μm was imaged by rastering the stage stepwise

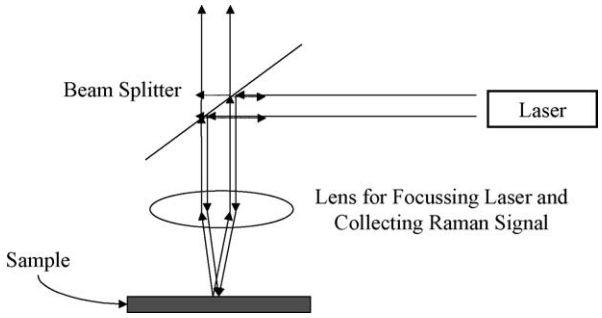

Fig. 1. Raman microscopy.

to obtain spot spectra. The step size in both the x and y was 1 μm (a total of 16,384 spectra were collected). Accumulation time of 10 s per spectrum was used which provided sufficient quality spectra for differentiation of the drug components (total collection time approximately 48 h).

On completion of the analysis, a "spectral image" file containing each of the 16,384 spectra was saved to disk, which were used to obtain the Raman images.

2.5. Manual thresholding

Manual thresholding was performed to obtain a rough graphical assessment of the location of the drug components in the field of view. Discrete diagnostic peaks for both fluticasone propionate and salmeterol were identified. Fig. 2 shows that for fluticasone propionate a sharp peak at 1670 $cm^{-1}$was used and integration of the peak occurred between 1650 and 1690 cm^{-1}.

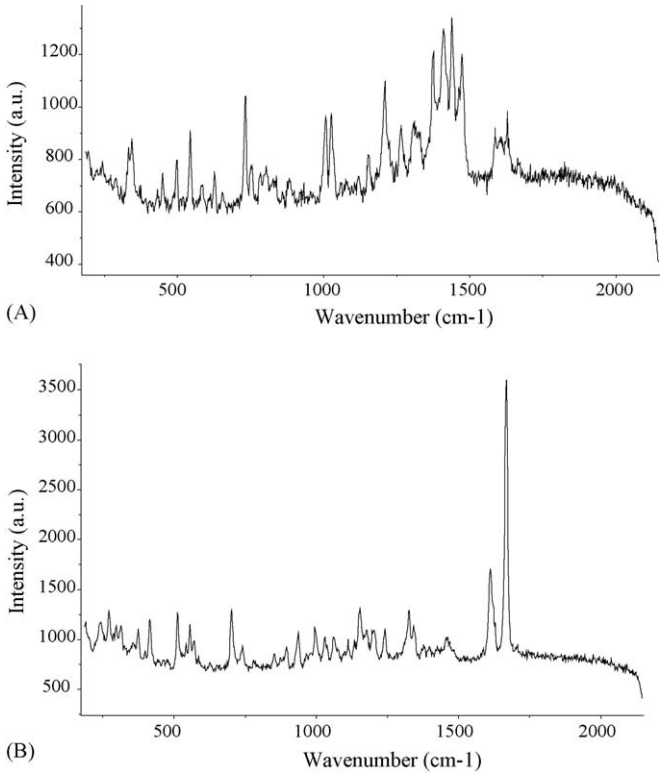

Fig. 2. Spectrum of salmeterol (A) and fluticasone propionate (B).

For salmeterol, a group of four peaks in the range 1380 and 1480 cm^{-1} were used and integration occurred between 1360 and 1500 cm^{-1}. The diagnostic peaks were baselined and integrated using the LabSpec software and plotted graphically as an image.

The data were thresholded to a binary image; i.e., pixels were only coloured on the image if the integrated value for that pixel was above a manually set threshold value. The threshold value was adjusted until an image showing a distribution of particles roughly equivalent to those seen in the stored optical image was obtained (Fig. 3). The output of this part of the process was a Raman image in which the pixels containing FP fluticasone propionate were coloured green, the pixels containing salmeterol were coloured red, and pixels containing both fluticasone propionate and salmeterol were coloured yellow. An overlay of the Raman image and the optical image was also obtained (Fig. 3). The majority of particles seen on the optical image were detected by Raman. However a small number of particles were not detected as a result of being beyond the 3.2 μm confocal depth of field.

2.6. Statistical thresholding

To remove the operator effect across samples, a statistical thresholding procedure was also applied. Presence of a drug was established when the intensity of the signal was considered to be different from random variation. The precision of the signal measurement was affected by the size of the peak relative to the noise. Estimates of the noise came from a baseline region of the spectrum. First differencing of the spectra (computing successive differences between consecutive values along the spectrum) was performed on the baseline region for each pixel. The standard deviation of the noise was estimated using the median absolute deviation of the first differenced baseline series (Miller and Miller, 1993). The signal measure was then standardised by the estimated standard deviation of the error. A threshold of 5 standard deviations for the major peaks of fluticasone propionate and 3 for all four peaks of salmeterol was set as the limit over which the standardised values were considered positive. A lower threshold was chosen for salmeterol because of its poor scattering properties. Images produced via statistical thresholding were similar to those obtained using a manual threshold, but did not rely on operator adjustments.

2.7. Measure of co-deposition

A measure of association (co-deposition) of the particles of salmeterol and fluticasone propionate, the Jaccard coefficient, was computed from statistically thresholded Raman images (Sokal and Sneath, 1963). The Jaccard coefficient is a value between 0 and 1, where values closer to one indicate that the two drugs co-deposit more in one image.

2.8. Bootstrap approach—within image approach

To assess whether the co-association of salmeterol and fluticasone propionate seen within the Raman images, either separate

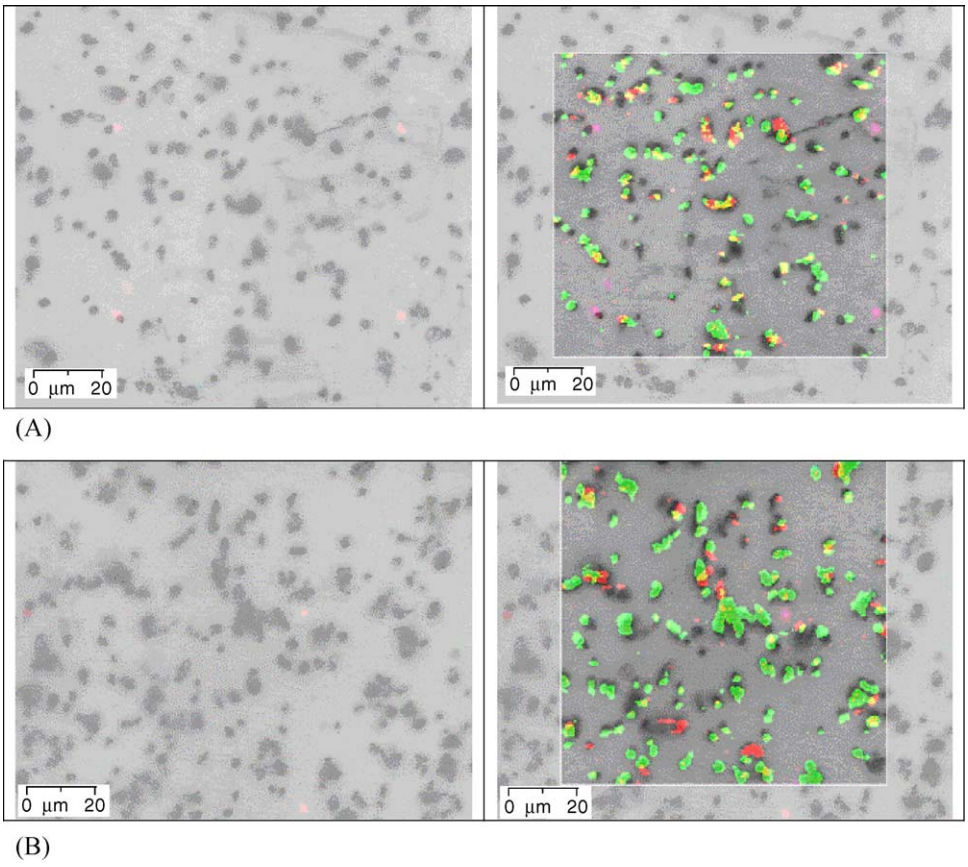

Fig. 3. Example optical image (left) and optical image plus manual threshold Raman overlay (right) of COMBINATION administration (A) and SEPARATE INHALER administration (B). Fluticasone propionate is green, salmeterol is red and any co-deposition is in yellow. Black means that neither of the drugs were detected.

or combined, was greater than by chance alone, a statistical technique termed bootstrapping (Davison and Hinkley, 1997) was used. A block-bootstrap technique originally proposed for time series was adapted to the Raman data. The bootstrap procedure randomly sub-samples the observed data from the Raman image to construct "bootstrap images" that retain the essential characteristics of the original images but assumes no co-association. Hence, any co-deposition that occurs between salmeterol and fluticasone propionate could be considered random. The spatial bootstrap procedure for a single image is depicted in Fig. 4. A resampling block was chosen (16 × 16 pixels) and a set of random pixel locations for a corner of the resampling block was generated to reconstruct an image with the same dimensions as the original Raman image. For a 16 × 16 resampling block on a 128 × 128 image, this involved 64 random locations. These resampled blocks of data were organised into a matrix creating a random image. This procedure was applied to fluticasone propionate and salmeterol independently. The degree of association

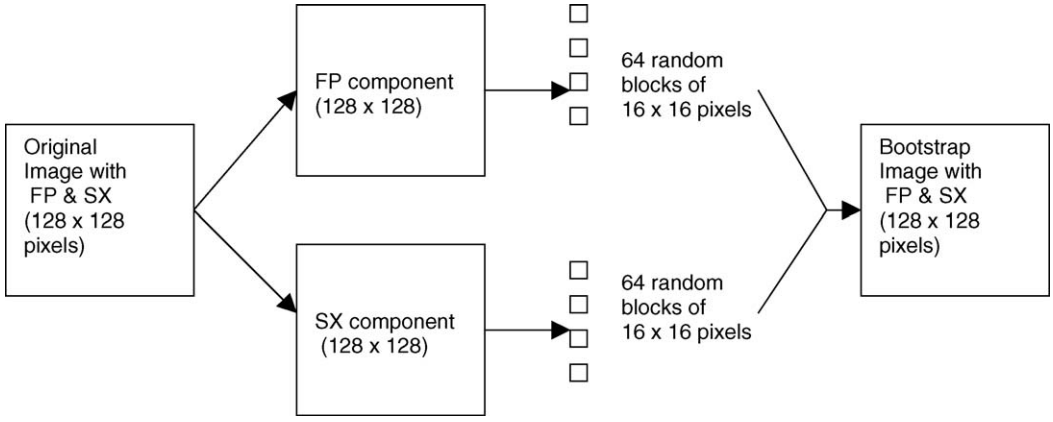

Fig. 4. Diagrammatic representation of the bootstrap technique.

(the Jaccard coefficient) was computed. This procedure was repeated 5000 times to produce a bootstrap distribution for the Jaccard coefficient assuming random co-deposition. A comparison of the true Jaccard coefficient computed from the original observed Raman data, to the bootstrap distribution was then performed to see if the level of association observed between salmeterol and fluticasone propionate could be explained by random variation.

2.9. Bootstrap approach—combined drug and separate drug administration

To determine if the level of co-deposition between salmeterol and fluticasone propionate differed between combined and separate drug administration, the measured association between the drugs in the COMBINATION inhaler and the SEPARATE INHALERS was compared. First, the difference between the

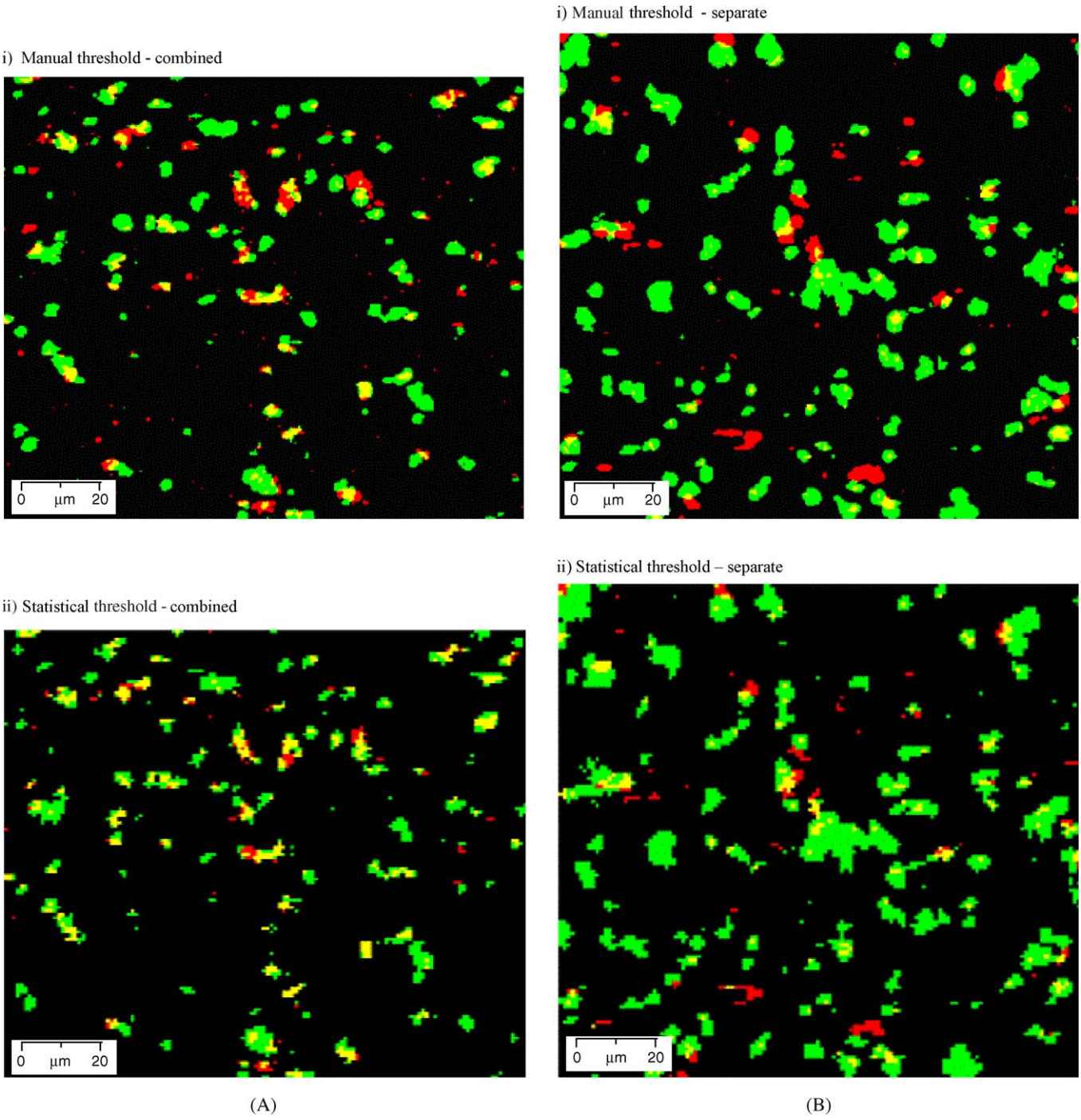

Fig. 5. Raman image produced by (i) manual and (ii) statistical thresholding of COMBINATION administration (A) and SEPARATE INHALER administration (B).

Jaccard coefficients for the COMBINATION and SEPARATE INHALERS pooled across the four images for each type of drug administration, was calculated. All eight images were then pooled to create one single image. The pooled data was resampled using 16 × 16 blocks, which was split into two groups (one labelled 'COMBINATION' and one labelled 'SEPARATE'), the Jaccard coefficient computed for each group of resampled data and the difference computed. This differs from the within image analysis because here both drugs were resampled using the same spatial pattern, whereas the drugs were sampled independently before. Performing these steps 2000 times produced a bootstrap distribution for the difference in the Jaccard coefficient, meaning that there was no difference in association between combined and separate drug administration. This technique is similar to applying the bootstrap to the classical two-sample *t*-test.

3. Results

3.1. Raman images

A sample of COMBINATION and SEPARATE INHALER drug applications collected from plate four of the cascade impactor is shown in Fig. 3. These images are optical images with the corresponding manual threshold Raman overlay images. These same images with the drug deposition defined by both manual and statistical thresholding for the COMBINATION and SEPARATE INHALERS are shown in Fig. 5. Salmeterol is coded red, fluticasone propionate is green and any co-deposition is in yellow.

3.2. Measure of association

The measure of association between fluticasone propionate and salmeterol calculated using the Jaccard coefficient from each of the four Raman images of particles collected on Plate 4 of the Andersen Cascade Impactor following each of the COMBINATION and SEPARATE INHALER actuations is shown in Table 1. While the Jaccard coefficients are clearly different between the COMBINATION and SEPARATE INHALER drug images, with more co-association apparent in the COMBINATION drug images (values closer to 1), there appears to be association between fluticasone propionate and salmeterol in all images regardless of whether the drugs were administered separately or combined.

Table 1
Coefficients for each image from COMBINATION and SEPARATE INHALERS

Data set	Jaccard
COMBINATION	
ra0579	0.217
ra0580	0.280
ra0582	0.154
ra0584	0.142
SEPARATE	
ra0577	0.059
ra0581	0.090
ra0583	0.053
ra0585	0.064

3.3. Bootstrap approach—within image

The bootstrap procedure was performed within each Raman image. The resulting bootstrap distribution for the Jaccard coefficient under the assumption of no co-association between salmeterol and fluticasone propionate is shown in Fig. 6 for two of the eight Raman images. It is clear that the observed level of association is outside the bootstrap distribution for the COMBINATION and SEPARATE INHALER drug images suggesting that in both cases the level of co-deposition was greater than by chance. This was true for all eight Raman images. However, the difference in co-deposition between the COMBINATION and SEPARATE INHALER cannot be determined using this analysis.

3.4. Bootstrap approach—combined drug and separate drug administration

The bootstrap distribution for the difference of Jaccard coefficients between the COMBINATION and SEPARATE

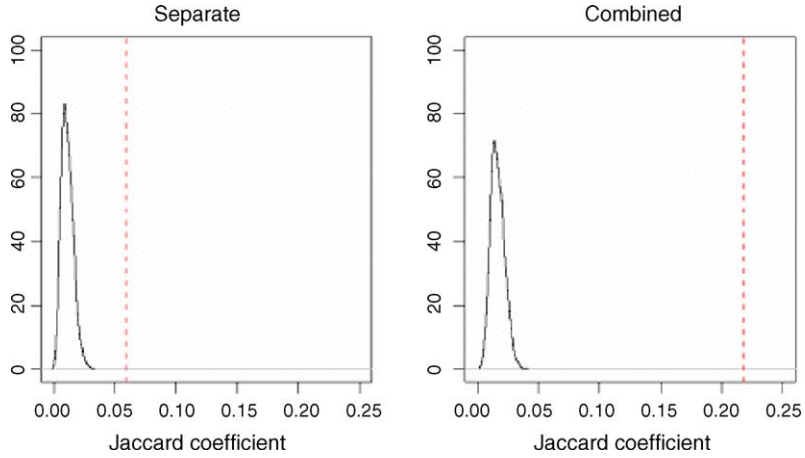

Fig. 6. An example of the observed Jaccard coefficients (vertical red lines) and bootstrap distributions under the assumption of independence between salmeterol and fluticasone. SEPARATE INHALER administration images are on the left and COMBINATION images on the right.

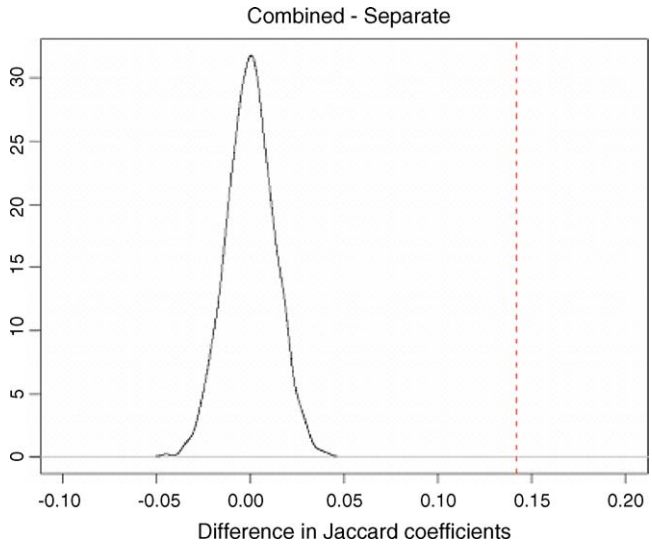

Fig. 7. Bootstrap distribution for the difference in Jaccard coefficients under the assumption of no difference between SEPARATE INHALER and COMBINATION images. The observed difference is given by the vertical (red) line.

INHALER drug application, under the assumption that the COMBINATION and SEPARATE INHALER images exhibit the same amount of co-deposition is shown in Fig. 7. The vertical (red) line is the observed difference between the pooled SEPARATE INHALER and COMBINATION images. It is clear that the observed difference is outside the bootstrap distribution i.e., co-deposition of salmeterol and fluticasone propionate is significantly greater when delivered from a COMBINATION inhaler versus administration via SEPARATE INHALERS ($p < 0.001$).

4. Discussion

A meta-analysis of four pivotal studies resulted in a greater proportion of patients achieving a clinically significant improvement in morning peak expiratory flow (demonstrated by the significant increase in the odds ratio of achieving an improvement of either >15 or >30 L/min) with Seretide when compared to the two-component drugs given concurrently from separate dry powder inhalers (Nelson et al., 2003). These differences were shown in controlled double-blind, double-dummy studies where any benefits arising from increased compliance with the single inhaler over two concurrent inhalers could not be achieved. Recent data has also shown that Seretide works synergistically to inhibit both the early and late phases in response to antigen challenge (Swenson et al., 2003) and to increase lung function in asthmatic patients (Aggarwal et al., 2003).

Several in vitro complementary mechanisms between ICS and LABAs have been demonstrated. At the receptor level, corticosteroids increase β2-adrenergic receptor transcription in human lung (Mak et al., 1995) and increase the synthesis of respiratory mucosal β2-receptors (Baraniuk et al., 1997). LABAs, like salmeterol, have been shown to prime the inactive glucocorticoid receptor, rendering the receptor more sensitive to steroid-dependent activation (Adcock et al., 2002; Johnson et al., 2002; Roth et al., 2002). These data suggest that there is more than one complementary mechanism involved in the simultaneous use of salmeterol and fluticasone propionate which would be enhanced by the significant co-deposition of the two drugs in the airways. In vitro analyses were therefore performed to determine if this synergy could be explained by a higher degree of co-deposition from drugs in the Seretide formulation compared with the component products given concurrently. Data from the Raman images showed that the co-deposition of fluticasone propionate and salmeterol from Seretide was significantly greater than the co-deposition when they were administered concurrently. This suggests that the particles within Seretide MDI co-associate and travel together leading to increased co-deposition in the airways. Hence the enhanced clinical effect from the combination of salmeterol and fluticasone propionate, in Seretide, compared to inhalation from separate inhalers may possibly be explained by greater co-deposition of the two drugs.

Previous studies have shown that there is no systemic pharmacokinetic interaction between salmeterol and fluticasone propionate when administered in combination (Kirby et al., 2000). It has also been shown that the amount of drug delivery of the combination is comparable to devices containing the individual drug products with respect to the fine particle mass of both salmeterol and fluticasone propionate and to the particle size distribution of the emitted dose (Ashurst et al., 1998; Malley et al., 1998). Greater total drug delivery with the combination product is therefore not responsible for the greater clinical benefits.

A novel adaptation of statistical methodology was used to compare the level of association between drugs in the Seretide formulation and concurrent administration of the individual drugs. Using the Jaccard coefficient to measure association between fluticasone propionate and salmeterol, it was found that the combination product had a greater level of association than in the images from separate administration of the drugs. Computing the measure of association between the salmeterol and fluticasone propionate on a cascade impactor plate is a good way to evaluate the amount of co-deposition on the plate, but it does not provide insight into the likelihood of observing that level of association. Since it is not possible to know everything about the physical process of the drug particles falling on the cascade impactor plates, the bootstrap technique was used (Davison and Hinkley, 1997). It is assumed that the drugs land on the plate independently of each other, but with some degree of spatial association between particles from the same drug. The spatial bootstrap procedure was performed within each Raman image and, although the Jaccard coefficients were clearly different between the combination and separate drug images, there appeared to be some association between fluticasone propionate and salmeterol in all images regardless of the method of administration. Calculating the Jaccard coefficients separately provided evidence that the combination product showed increased co-deposition, but a statistical hypothesis test was needed to validate the results; i.e., was the association measured between the drugs different in combined versus separate administration? In this analysis, both drugs were re-sampled using the same spatial pattern, whereas the drugs were sampled independently in the within-image analysis. By applying a non-parametric bootstrap to the classical two-sample *t*-test (for a difference in the average Jaccard coefficient of the two samples) a significant difference

was found hence there was evidence of co-association between drug particles in the combination sample when compared to separate drug administration.

The obvious limitation of this analysis is that it has been observed in vitro using an Andersen Cascade Impactor. This apparatus is commonly utilised to determine drug deposition characteristics in vitro (British Pharmacopoeia, 2002). We chose to look at the possible degree of association between fluticasone propionate and salmeterol on Plate 4 of the impactor which is generally thought to be representative of deposition in the central airways. However, in vivo, the difference would be anticipated to be much greater than in vitro. With the extent of mass of branching of the airways, the likelihood of particles co-depositing by chance rather than by co-association are greatly reduced.

The droplet size from a MDI immediately after actuation is approximately 20–50 μm. This size starts to reduce instantaneously due to evaporation of the propellant. Since the particle size of the drug input material is less than 3 μm then it is highly probable that the initial droplets may contain more than one particle. It is also likely that these particles within a droplet have the tendency to stay together once the propellant evaporates. The evaporation of the propellant is an incredibly rapid process and will be completed by the time the particle impacts in the Andersen Cascade Impactor or is deposited into the lungs. Furthermore it has been previously demonstrated that there is an interaction between salmeterol and fluticasone propionate drug particles within an aerosol propellant system, with a tendency of the two drugs to form particle agglomerations within the inhaler (Michael et al., 2001). This has been verified by the significant co-association of salmeterol and fluticasone propionate drug particles seen in this in vitro analysis on the cascade impactor plate, which may provide an explanation for the enhanced efficacy seen in clinical studies. It is possible that the co-association of particles seen in the MDI formulation might be as a result of evaporation of the carrier propellant during inhalation which may not happen following inhalation from the Diskus which contains lactose particles as the carrier. However, the increased clinical benefit is observed from studies using the Diskus and there is evidence that the interaction is likely to also occur in dry powder systems (Young et al., 2004). Although the clinical studies showing increased efficacy were conducted using a dry powder inhaler and this in vitro analysis performed with the MDI, bio-equivalence between the MDI and Diskus has been demonstrated in numerous studies (Bateman et al., 2001; van Noord et al., 2001; Emeryk et al., 2003). The MDI was used for practical reasons to simplify analysis of the Raman data since only salmeterol and fluticasone propionate particles are present. For optimal interaction between the two drugs, they must reach the same target cell together in adequate concentrations. It is assumed from these results that in patients, there would be co-deposition of salmeterol and fluticasone propionate at β_2 and glucocorticoid receptors on the same cell when the two drugs are administered in combination. This is in contrast to the pattern of deposition when the two drugs are administered from separate devices, where the likelihood of drug co-deposition by chance on the same cell would be lower following successive inhalations given the degree of branching in the airways. The molecular ratio of salmeterol and fluticasone propionate may contribute to this being a specific and not a generally applicable finding to all combination products.

5. Conclusion

This analysis has demonstrated that there is significant co-association of salmeterol and fluticasone propionate particles, leading to increased co-deposition when they are administered from the same inhaler. This provides a greater opportunity for a synergistic interaction between the two drugs to occur in the airways and may possibly be a significant factor contributing to the enhanced clinical effect seen in comparison with that observed when the drugs are administered separately from two inhalers.

Acknowledgements

We would like to thank Renata Williams for her help in generating the cascade impactor plates for analysis. We would also like to thank Diana Jones with her help in the preparation of this manuscript.

References

Adcock, I.M., Maneechotesuwan, K., Usmani, O., 2002. Molecular interactions between glucocorticoids and long-acting beta2-agonists. J. Allergy Clin. Immunol. 110, S261–S268.

Aggarwal, S.K., Frith, L.J., Ho, M., Weeks, T.C., Ho, S.Y., 2003. Fluticasone propionate/salmeterol delivered from a single inhaler demonstrates synergistic benefits in asthma. Am. J. Respir. Crit. Care Med. 167, A890.

Andersen Samplers Inc. Operating Manual for Andersen 1 ACFM Non-Viable Ambient Particle Sizing Samplers, 1985. Published by Andersen Samplers Inc. Atlanta, GA, USA.

Ashurst, I.C., Prime, D., Haywood, P.A., 1998. Development of a dry powder inhaler device containing a combination of salmeterol and fluticasone propionate. Eur. Respir. J. 12, 93s.

Aubier, M., Pieters, W., Schlosser, N., Steinmetz, K.-O., 1999. Salmeterol/fluticasone propionate (50/500 mcg) in combination on a Diskus inhaler (Seretide) is effective and safe in the treatment of steroid dependent asthma. Respir. Med. 93, 876–884.

Baraniuk, J.N., Ali, M., Brody, D., Maniscalco, J., Gaumond, E., Fitzgerald, T., Wong, G., Yuta, A., Mak, J.C., Barnes, P.J., Bascom, R., Troost, T., 1997. Glucocorticoids induce beta2-adrenergic receptor function in human nasal mucosa. Am. J. Respir. Crit. Care Med. 155, 704–710.

Barnes, P.J., 2002. Scientific rationale for inhaled combination therapy with long-acting beta2-agonists and corticosteroids. Eur. Respir. J. 19, 182–191.

Bateman, E.D., Britton, M., Carrillo, J., Almeida, J., Wixon, C., 1998. Salmeterol/fluticasone (50/100 mcg) combination inhaler. A new, effective and well-tolerated treatment for asthma. Clin. Drug Invest. 16, 193–201.

Bateman, E.D., Silins, V., Bogolubov, M., 2001. Clinical equivalence of salmeterol/fluticasone propionate in combination (50/100 mcg twice daily) when administered via a chlorofluorocarbon-free metered dose inhaler or dry powder inhaler to patients with mild-to-moderate asthma. Respir. Med. 95, 136–146.

British Pharmacopoeia, 2002. General Monographs, Preparations for Inhalation.

British Guideline on the Management of Asthma, 2004. Update April 2004. http://www.brit-thoracic.org.uk/.

Chapman, K.R., Ringdal, N., Backer, V., Palmqvist, M., Saarelainen, S., Briggs, M., 1999. Salmeterol and fluticasone propionate (50/250 mcg) administered via combination Diskus inhaler: as effective as when given via separate Diskus inhalers. Can. Respir. J. 6, 45–51.

Davison, A.C., Hinkley, D.V., 1997. Bootstrap Methods and their Applications. Cambridge University Press, Cambridge, England.

Emeryk, A., Bracomonte, T., Schauer, U., Balsara, S., Athisegaran, R., 2003. Efficacy of salmeterol/fluticasone propionate (50/100 mcg/day) delivered via the Diskus and MDI in children with asthma. Eur. Respir. J. 22, 1985.

Fraser Steele, D., Young, P.M., Price, R., Smith, T., Edge, S., Lewis, D., 2004. The potential use of Raman mapping to investigate deposition of combination pressurized metered-dose inhalers. AAPS J. 6, Article 21 (http://www.aapsj.org).

Global Initiative for Chronic Obstructive Lung Disease (GOLD), 2003. Publication no. 2701. National Institutes for Health, Bethesda, 2001 (2003 update).

Greening, A.P., Ind, P., Northfield, M., Shaw, G., 1994. Added salmeterol versus higher-dose corticosteroid in asthma patients with symptoms on existing inhaled corticosteroid. Lancet 344, 219–224.

Guidelines for the Diagnosis and Management of Asthma, 1997. Expert panel report 2. National Institutes of Health, National Heart, Lung and Blood Institute, July 1997. NIH publication number 97-4051.

Johnson, M., 2002. Combination therapy for asthma: complementary effects of long-acting ß2-agonists and corticosteroids. Curr. Allergy Clin. Immunol. 15, 16–22.

Kirby, S., Falcoz, C., Daniel, M.J., Milleri, S., Squassante, L., Ziviani, L., Ventresca, G.P., 2000. Salmeterol and fluticasone propionate given as a combination: lack of systemic pharmacodynamic and pharmacokinetic interactions. Eur. J. Clin. Pharmacol. 56, 781–791.

Mak, J.C., Nishikawa, M., Barnes, P.J., 1995. Glucocorticosteroids increase B2-adrenergic receptor transcription in human lung. Am. J. Physiol. 268, L41–L46.

Malley, T.R., Ashurst, I.C., Daniels, G.E., Prime, D., Stock, N.J., Haywood, P.A., 1998. Fine particle mass of a dry powder inhaler device containing a combination of salmeterol and fluticasone propionate. Eur. Respir. J. 12, 8s.

Matz, J., Emmett, A., Rickard, K., Kalberg, C., 2001. Addition of salmeterol to low-dose fluticasone versus higher-dose fluticasone: an analysis of asthma exacerbations. J. Allergy Clin. Immunol. 107, 783–789.

Michael, Y., Snowden, M.J., Chowdhry, B.Z., Ashurst, I.C., Davies-Cutting, C., Riley, T., 2001. Characterisation of the aggregation behaviour in a salmeterol and fluticasone propionate inhalation aerosol system. Int. J. Pharm. 221, 165–174.

Miller, J.C., Miller, J.N., 1993. Statistics for Analytical Chemistry, 3rd ed. Ellis Horwood PRT Prentice Hall, Analytical Chemistry Series.

Nelson, H., Chapman, K.R., Pyke, S.D., Johnson, M., Pritchard, J.N., 2003. Enhanced synergy between fluticasone propionate and salmeterol inhaled from a single inhaler versus separate inhalers. J. Allergy Clin. Immunol. 112, 29–36.

Roth, M., Johnson, P.R., Rudiger, J.J., King, G.G., Ge, Q., Burgess, J.K., Anderson, G., Tamm, M., Black, J.L., 2002. Interaction between glucocorticoids and beta2 agonists on bronchial airway smooth muscle cells through synchronised cellular signalling. Lancet 360, 1265–1266.

Shrewsbury, S., Pyke, S., Britton, M., 2000. Meta-analysis of increased dose of inhaled steroid or addition of salmeterol in symptomatic asthma (MIASMA). Br. Med. J. 320, 1368–1373.

Sokal, R.R., Sneath, P.H., 1963. Principles of Numerical Taxonomy. W.H. Freeman, San Francisco.

Swenson, C., Laseman, J., Busse, W., 2003. Combination of salmeterol (SAL) and Fluticasone (FP) works synergistically to inhibit airway inflammation post allergen challenge. J. Allergy Clin. Immunol. 111, S126.

Van den Berg, N.J., Ossip, M., Hederos, C.A., Anttila, H., Ribeiro, B.L., Davies, P.I., 2000. Salmeterol/fluticasone propionate (50/100 mcg) in combination in a Diskus inhaler (Seretide) is effective and safe in children with asthma. Paediat. Pulmonol. 30, 97–105.

van Noord, J.A., Lill, H., Carrillo Diaz, T., Greefhorst, A.P., Davies, P., 2001. Clinical equivalence of salmeterol/fluticasone propionate combination product (50/500 g) delivered via a chlorofluorocarbon-free metered dose inhaler with the Diskus in patients with moderate to severe asthma. Clin. Drug Invest. 21, 243–255.

Woolcock, A., Lundback, B., Ringdal, N., Jacques, L.A., 1996. Comparison of addition of salmeterol to inhaled steroids with doubling of the dose of inhaled steroids. Am. J. Respir. Crit. Care Med. 153, 1481–1488.

Young, P.M., Price, R., Jones, S., Parry-Billings, M., 2004. Investigation into drug and excipient interaction in dry powder inhaler combination products. Proc. Respir. Drug Deliv. IX 3, 749–752.

Zetterström, O., Buhl, R., Mellem, H., Perpiñà, M., Hedman, J., O'Neill, S., Ekstrom, T., 2001. Improved asthma control with budesonide/formoterol in a single inhaler, compared with budesonide alone. Eur. Respir. J. 18, 262–268.