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Equivalence testing of salbutamol dry powder inhalers: in vitro impaction results versus in vivo efficacy

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Abstract

The aim of the study was to evaluate several impactors for in vitro equivalence testing of salbutamol with respect to efficacy and to define in vitro equivalence limits validated with in vivo efficacy data. The four impactors described in Supplement 2000 of the European Pharmacopoeia were used: Glass Impinger (GI), Metal Impinger (MI), Multistage Liquid Impinger (MSLI) and Andersen Cascade Impactor (ACI). Three salbutamol dry powder formulations with different fine particle doses (FPDs) were prepared and the aerodynamic particle size distribution was measured. For each impactor also the recovery was determined. The same three preparations were administered to 12 asthmatic patients in a randomized, placebo-controlled, four-way crossover study. Cumulative doses from 50 μg up to 400 μg were given. The FEV_1 was measured at baseline and 15 min after each dose. The in vitro results showed large differences between the FPDs of the three preparations with all impactors, whereas only small differences were observed between the four impactors. Since the recoveries of the MI and GI were low, in vitro equivalence testing should only be performed with the MSLI or ACI. The in vivo measurements did not show significant differences in efficacy between the three active preparations, even at the most discriminatory dose of 50 μg. It is concluded that in case there are no relevant differences between delivered dose, inhalation device and excipients, for salbutamol dry powder inhalers equivalence can be assumed when the 90% confidence interval for the FPD ratio of the test product and reference product is within 0.50–1.20 and each of the two products has a FPD (particles <6 μm) of at least 10 μg.

Keywords: Equivalence; Salbutamol; Dry powder inhaler; Impactor; Fine particle dose; Efficacy

1. Introduction

Comparison of the therapeutic performances of medicinal products is important for assessment of their interchangeability. Two products are ther-

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apeutically equivalent if they contain the same active substance and have the same efficacy and safety. For inhalation products several methods for equivalence testing are available (Snell, 1997). Comparative clinical trials are considered as the 'golden standard', but the statistical proof of equivalence is hampered by the inter- and intra-individual variability of the anti-asthmatic effects (Zanen and Lammers, 1995). Pharmacodynamic studies could be used as an alternative approach, but appropriate tests are not always available (CPMP, 1995). Furthermore, there is no consensus on the endpoints to be used and clearly defined clinically relevant differences or equivalence limits are lacking.

Since the clinical response of inhalation products will be determined by the amount of drug available on the site of action, comparative lung deposition studies with radiolabelled products are used as evidence for equivalence (Newman, 1998). Pharmacokinetic studies can be applied as an alternative method to estimate lung deposition (Chege and Chrystyn, 2000). A major disadvantage of the latter two methodologies is the lack of validation with respect to their correlation to clinical effects and, again, clinically relevant differences or equivalence limits are not defined.

It is generally believed that lung deposition of inhaled particles depends on the aerodynamic particle size distribution, which can be measured in vitro by impactors. The relative ease of operation, the high power to detect differences and the relatively low variability in the measurements compared to in vivo experiments make in vitro methods attractive. In a previous study, it was discussed under which circumstances comparative in vitro equivalence testing is allowed (Weda et al., 2000). It has been suggested by Weda et al. that in case the delivered dose of the test product differs not more than 20% of the delivered dose of the reference product, the inhalation device is identical and any qualitative or quantitative difference in excipients does not influence the inhalation behaviour of the patient, in vitro impaction data may be used to provide evidence for equivalence of two inhalation products. It was also postulated that the 90% confidence interval (CI) for the in vitro deposition ratio of the two products should lay within 0.80–1.20. This range was challenged by applying it to marketed inhalation products and appeared to be prudent, but rather strict. In addition, the challenge was only applied to deposition results obtained with the Twin Impinger. Furthermore, a direct comparison of the in vitro deposition results and the clinical effects was not made. It is not known what differences in aerodynamic particle size distribution become clinically relevant. The proposed equivalence range is therefore not fully validated.

The aim of this study was to evaluate the use of impactors for in vitro equivalence testing and to validate the earlier proposed in vitro equivalence limits for salbutamol dry powder inhalers. The four impactors described in Supplement 2000 of the European Pharmacopoeia (EP; European Pharmacopoeia, 1997a) were included in this study.

2. Materials and methods

2.1. Preparations

Three salbutamol dry powder formulations with different fine particle doses (FPDs) were manufactured by the Pharmaceutical Technology and Biopharmacy Department of the University of Groningen, The Netherlands. Micronized salbutamol sulphate ($X_{90} = 3.4$ μm, $X_{50} = 1.4$ μm and $X_{10} = 0.7$ μm) was used as active substance and was granted by Genfarma B.V., The Netherlands. Sieve fractions from lactose monohydrate 80 Mesh (250–315 μm) and 100 Mesh (90–106 μm) were used as carrier excipient for the preparation of adhesive mixtures. These preparations were assigned as coarse (COARSE) and intermediate (INTER), having low and intermediate FPDs respectively. Micronized lactose ($X_{50} = 3.4$ μm) was used as excipient in the preparation of spherical pellets. This preparation was assigned as fine (FINE), having the highest FPD.

The adhesive mixtures were prepared by mixing lactose and salbutamol sulphate in a stainless steel container during 10 min in a tumbling mixer at 90 rpm (Turbula T2C, W.A. Bachofen AG, Switzerland). The spherical pellet preparation was man-

ufactured by homogenizing, densification and pelletizing of the salbutamol and micronized lactose blend in the same stainless steel mixing container (10 min at 90 rpm), using stainless steel beads with a well defined size distribution as pelletizing aid. Next, the fines were removed and the remaining pellets were spheronized on a 200 μm vibratory sieve for 20 min (Fritsch Analysette 3, Fritsch GmbH, Germany). Finally, the pellets were classified into different size fractions by mild hand sieving; the fraction 315–800 μm was used for the experiments. For each preparation strengths of 50 and 100 μg salbutamol base per actuation were made.

The dry powder formulations were filled into a bulk container of a Novolizer® multi-dose dry powder inhaler having an intermediate air flow resistance of 0.028 $kPa^{0.5}$/min/l (Sofotec, Germany; Berner et al., 1998). A placebo was prepared from lactose monohydrate 80 Mesh. Before release by the responsible pharmacist, the quality of the preparations was checked by the Laboratory for Quality Control of Medicines of the National Institute for Public Health and the Environment, The Netherlands. The dose uniformity of all preparations was amply within pharmacopoeial standards, with relative standard deviations of 0.7% up to 1.6%. The preparations were stored at 18 °C and 32% relative humidity.

2.2. Impactors

The aerodynamic particle size distribution of the active preparations was measured with the Glass Impinger (GI), Metal Impinger (MI), Multistage Liquid Impinger (MSLI) and the Andersen Cascade Impactor (ACI), according to the instructions of the EP (Table 1). The throats used were also according to the EP, i.e. glass throat for the GI, metal throat for the MI, and the USP throat for the MSLI and ACI. For the MSLI a pressure drop across the inhaler of 4.0 kPa was applied, resulting in a flow rate of 80 l/min. For the ACI the maximum achievable flow rate of 60 l/min was used, corresponding to 2.3 kPa across the inhaler. The impaction plates of the ACI were coated with 2% viscous oil in hexane. The GI and MI were also operated with a flow rate of 60 l/min. The theoretical cut-off diameters at these flow rates are shown in Table 1 (European Pharmacopoeia, 1997a; Olsson et al., 1996; Nichols, 2000). The cut-off diameter for the FPD is determined by the stage with the closest cut-off diameter to 5.0 μm.

2.3. In vitro measurements

Ten actuations of the 50 μg preparations and five actuations of the 100 μg preparations were delivered to the apparatus. The amounts of salbutamol in the mouthpiece of the Novolizer® inhaler, the mouthpiece adaptor, the impactor throat and the impactor stages were determined with HPLC using a 125×4 mm^2 Hypersil BDS 5 μm C18 column with guard column, a detection wavelength of 278 nm and methanol:water 15:85 v/v as eluent. This method was validated with respect to accuracy, precision, linearity, range and quantification limit and proved to be suitable according to internal GLP standards.

For all active preparations the FPD in each impactor was determined. The measurements were performed in triplicate. The delivered dose was

Table 1
Operating conditions and characteristics of the impactors

	GI	MI	MSLI	ACI
Flow rate (l/min)	60	60	80	60
Time per actuation (s)	5	5	3	4
Volume per actuation (l)	5	5	4	4
Stages for FPD determination[a]	2	2	3–5	1–8
Cut-off diameter for FPD (μm)	6.4	9.4	5.9	5.9

[a] Stage 2 of the GI is defined as the lower impingment chamber. Stage 2 of the MI is defined as thefilter assembly. Stage 5 and stage 8 of the MSLI and ACI are defined as the final collection filters.

calculated by addition of the amounts deposited in the mouthpiece adaptor, the impactor throat and the impactor stages. The metered dose was determined by measuring the difference in weight of the dry powder bulk reservoir before and after the deposition test and by multiplying this weight with the active substance content of the powder. The recovery was calculated by summation of the delivered dose and the fraction deposited in the mouthpiece of the Novolizer® and is expressed as percentage of the metered dose.

2.4. In vivo measurements

Twelve mildly to moderately asthmatic subjects (eight female; Table 2) participated in the study. All subjects were non-smokers and had stable asthma, controlled by β_2-agonists and/or corticosteroids by inhalation. One patient used a xanthine derivative. Each subject had a baseline FEV_1 of $>$ 70% of predicted (Quanjer et al., 1993) and a bronchodilator response of $>9\%$ of predicted after inhalation of 400 μg salbutamol. Except for bronchodilators all medication was continued. Short acting β_2-agonists, long acting β_2-agonists and xanthine derivatives were discontinued respectively 8, 12 and 24 h prior to each test session. The study was approved by the Ethic Committee of the St. Antonius Hospital Nieuwegein, The Netherlands. All patients gave their written consent to participation.

The in vivo study followed a randomised, placebo-controlled, four-way crossover design. On four separate sessions with intervals of 1 week the three active preparations and the placebo were administered with the Novolizer®. At each session subsequent doses of 50, 50, 100 μg and 2 × 100 μg were administered with a dosing interval of 20 min. Before starting each session the subjects were given detailed inhalation instructions. The inhalation manoeuvre consisted of a deep and forceful inhalation, followed by a breath-holding period of 10 s. The inhalation flow through the Novolizer® was recorded by measuring the pressure drop over the inhaler, from which the flow was calculated. A peak inspiratory flow of >60 l/min and an inhalation time of >2 s through the Novolizer® was necessary in order to start a session. At the beginning of each session and 15 min after each salbutamol administration the flow–volume curves were measured using a pneumotachograph. The FEV_1 was selected as main parameter. The baseline FEV_1 was not allowed to vary more than 10% between the sessions.

2.5. Statistical analysis

The FEV_1 is expressed as a percentage of the predicted value (Weir and Sherwood Burge, 1991) and plotted against the nominal dose. The dose–response curves of the three active preparations and the placebo were analysed by repeated measurements analysis of variance for preparation and dose differences and for a dose by preparation interaction. The 90% CI for the differences between the three active preparations was calculated.

The FPD is expressed in μg salbutamol base per actuation. The recovery is expressed as percentage of the metered dose. Per impactor the mean recovery was calculated and the significance of differences between the impactors was determined by analysis of variance.

For the FPD as well as the recovery, the equality of the variances of the impactors was tested using Bartlett's test in order to decide whether significant differences in variability are present between the four impactors.

In all calculations an α-value of 0.05 was considered to be significant.

Table 2
Patient characteristics

Characteristic	Age (yrs)	Height (cm)	Weight (kg)	FEV_1 (% pred)	Reversibility (% pred)
Mean ± S.D.	36.4 ± 13.1	172.6 ± 6.6	76.3 ± 22.0	88.7 ± 12.5	20.4 ± 9.5

Table 3
Fine particle dose, in μg salbutamol per actuation ± standard deviation (S.D.).

	50 μg			100 μg		
	COARSE	INTER	FINE	COARSE	INTER	FINE
GI	7.4 ± 1.1	8.2 ± 1.1	25.7 ± 1.4	11.9 ± 1.1	21.2 ± 2.7	57.2 ± 0.6
MI	6.5 ± 0.6	10.0 ± 0.2	27.5 ± 0.8	12.8 ± 1.2	21.0 ± 2.9	55.3 ± 2.7
MSLI	9.3 ± 0.6	11.1 ± 2.2	23.3 ± 1.1	15.7 ± 1.0	23.2 ± 4.8	50.8 ± 2.0
ACI	9.2 ± 0.5	11.0 ± 0.9	30.0 ± 0.8	15.6 ± 2.2	23.5 ± 4.6	60.6 ± 1.9

3. Results

3.1. In vitro results

The FPD results are shown in Table 3 and the recoveries are shown in Table 4. The mean recoveries of the four impactors are significantly different from each other ($P < 0.05$). No significant differences were observed between the variances of the impactors. For the MSLI and ACI, the complete deposition pattern of the 50 μg preparations is shown in Figs. 1 and 2, respectively. The deposition pattern of the 100 μg preparations is comparable and is therefore not included.

3.2. In vivo results

All patients completed the four sessions. The dose–response curves are shown in Fig. 3. Statistical evaluation showed significant differences between the placebo and the three active preparations ($P < 0.011$). The dose by preparation interaction was significant ($P < 0.001$), indicating that the dose–response curves for the placebo and the active preparations did not run parallel.

No significant differences were observed between the three active preparations. The difference between COARSE and INTER was 0.88% of the predicted FEV_1 (90% CI of −3.2–1.4%), between COARSE and FINE 1.3% of the predicted FEV_1 (90% CI of −4.3–1.7%) and between INTER and FINE 0.43% of the predicted FEV_1 (90% CI of −3.1–2.3%).

4. Discussion

For the in vitro experiments all apparatus described in Supplement 2000 of the EP were used (European Pharmacopoeia, 1997a). The GI, MI and MSLI were fully operated according to the instructions for dry powder inhalers included in the EP. With respect to the ACI, a flow rate of 28.3 l/min is not appropriate for the Novolizer®. This flow rate corresponds with a pressure drop of only 1.7 kPa, which is unrealistic for a breath controlled DPI in relation to what patients are able to achieve through a device with an intermediate air flow resistance. However, due to the relatively high resistance of the ACI, it was not possible to obtain a pressure drop of 4.0 kPa

Table 4
Recovery, in percentage of the metered dose ± standard deviation (S.D.).

	50 μg			100 μg			
	COARSE	INTER	FINE	COARSE	INTER	FINE	Mean
GI	97.9 ± 1.7	96.1 ± 1.4	89.9 ± 1.5	99.2 ± 2.2	95.4 ± 1.3	91.4 ± 1.8	94.8
MI	92.9 ± 2.1	82.4 ± 0.5	81.1 ± 1.8	86.9 ± 0.9	85.6 ± 3.3	84.9 ± 3.7	85.6
MSLI	96.6 ± 2.1	99.5 ± 3.3	93.2 ± 1.1	98.9 ± 2.7	96.3 ± 1.6	96.8 ± 2.5	97.1
ACI	103.4 ± 1.3	98.9 ± 0.7	99.9 ± 1.6	101.4 ± 2.8	95.5 ± 3.0	98.2 ± 4.3	99.6

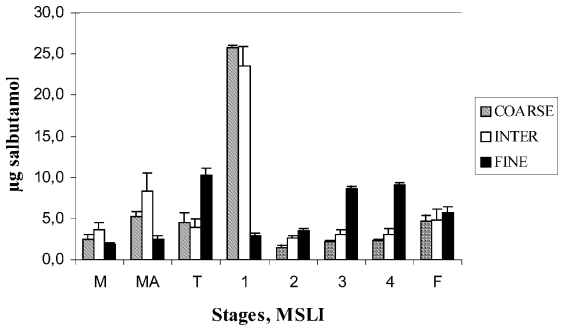

Fig. 1. Deposition pattern of the 50 µg preparations, obtained with the MSLI, including standard deviations. M, mouthpiece of the inhaler device; MA, mouthpiece adaptor; T, impactor throat; F, filter.

across the inhaler, as required for the MSLI. The maximum achievable flow rate of 60 l/min was therefore used.

In order to evaluate the four impactors for in vitro equivalence testing the variance and the recovery were taken into account, because an apparatus with an unfavourable variance and/or low recovery should not be used. In order to validate the in vitro equivalence limits the FPD was chosen as parameter, since the FPD comprises the size fractions with greatest potential to enter the lungs. It could be questioned whether the complete in vitro deposition pattern should be taken into consideration for equivalence testing, because the larger particles, deposited in the mouthpiece adaptor (MA), impactor throat (T) and upper impactor stages, could contribute to the

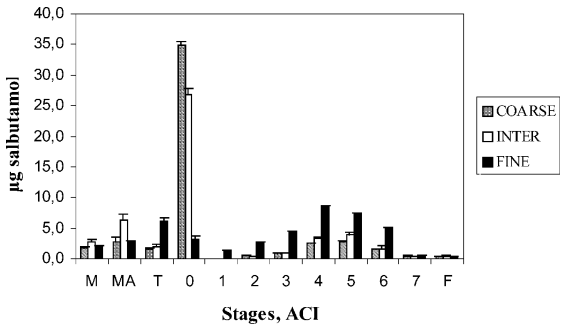

Fig. 2. Deposition pattern of the 50 µg preparations, obtained with the ACI, including standard deviations. M, mouthpiece of the inhaler device; MA, mouthpiece adaptor; T, impactor throat; F, filter.

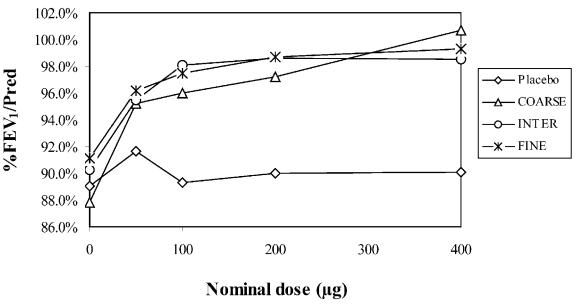

Fig. 3. Dose–response curve for salbutamol. The FEV_1 is expressed as precentage of predicted.

clinical effects and side effects due to gastrointestinal absorption. It is shown in Fig. 1 and Fig. 2 that for the COARSE and INTER preparations this large particle fraction (stages MA up to 2 and stages MA up to 1, respectively) is much higher than for the FINE preparation. Theoretically, this higher large particle fraction may compensate for the lower FPD. However, there was no difference in FEV_1 between all three preparations at 20 min (after dosing 50 µg) and 40 min (after dosing 100 µg). It is unlikely that at these timepoints the swallowed fraction of the preparations is already completely absorbed. Moreover, any absorbed salbutamol is subject to extensive first pass metabolism. Hence, the clinical effect must be the result of deposition in the lungs. For salbutamol no local side effects in the mouth and throat are to be expected. We therefore chose to use only the FPD for in vitro equivalence testing of salbutamol preparations.

The results for the FPD show marked differences between the COARSE, INTER and FINE preparations. These differences are comparable for each of the four impactors used. No significant differences were observed between the variances of the impactors. So, based on variability data no preference can be declared for any of the impactors. This conclusion is in line with the results obtained by Olsson et al., 1996, who evaluated the reproducibility of the GI, MI, MSLI and ACI for dry powder inhalers and pressurized metered dose inhalers and also found that all impactors had comparable reproducibility.

The mean recoveries of the impactors are significantly different from each other. For the

MI the recovery is considerably lower than for the other three apparatus and even falls below 90%. This means that a considerable amount of salbutamol is lost, presumably in the fine particle range, which can underestimate the FPD. It is also remarked that the GI recovery results for the preparation FINE are significantly lower than for the MSLI and ACI. The reason for the low recoveries of the MI and GI is unclear. For the MI this could be the result of the design of the apparatus (many connections and small cavaties). For the GI this could be due to the absence of a final filter. Due to these low recoveries the GI and MI cannot be considered as acceptable apparatus for aerodynamic particle size determination. Furthermore, it is generally recognised that only particles smaller than approximately 6 μm will be deposited in the lung (Pritchard, 2001). Due to the high cut-off diameter the MI is also from a theoretical point of view less suitable for in vitro equivalence testing. Indeed, the GI and MI are no longer prescribed for FPD determination in Supplement 2001 of the European Pharmacopoeia, 1997b. It is therefore concluded that the MSLI and ACI are the best candidates for in vitro equivalence testing, the GI and MI being less suitable.

For the in vivo study, 50 μg salbutamol was chosen as starting dose, because we wanted to be able to compare the active preparations at the steep part of the dose–response curve. The discriminatory power of the trial was further maximised by selecting reversible asthmatics. The study was sufficiently powerful. Enright et al. (1991), showed that the mean intra-individual standard deviation of the serially measured FEV_1 is 120 ml approximately. In our study the largest 90% CI width for the differences between the preparations was 3.0% of the predicted FEV_1, which is equivalent to 103 ml. The observed FEV_1 differences between the active preparations are therefore not considered as clinically relevant.

For the impactors used in the in vitro experiments the FPD of the preparation FINE was at least 2.5 times higher than the preparation COARSE. So, despite this large difference in FPD no significant differences in FEV_1 were observed between the three active preparations. In a previous study it was proposed that the 90% CI for the in vitro deposition ratio of two inhalation products should lay within 0.80–1.20 (Weda et al., 2000). Our data suggest that these limits are too strict for salbutamol in the tested dose range and can be widened, at least for in vitro equivalence testing of efficacy. For determination of revised equivalence limits only the in vitro results of the 50 μg preparation will be used, since the 50 μg is the most discriminatory dose with respect FEV_1 differences in the in vivo study and because this is the lowest strength currently on the market.

With the ACI, the lowest result for the FPD of the 50 μg preparation is 9.2 μg. This amount has been shown to have no significant difference in efficacy compared with a FPD of 30.0 μg. In other words, a test product with a FPD of 9.2 μg has the same efficacy as a reference product with a FPD of 30.0 μg. The FPD ratio of the test product COARSE and reference product FINE is 0.31. When taking into account the standard deviations of the FPDs, the upper limit of the 95% one-sided CI of the ratio is 0.33. For the MSLI, the same calculations reveal that the upper limit of the 95% one-sided CI of the ratio is 0.44. Combining these findings of the ACI and MSLI it is prudent to define 0.50 as the lower in vitro limit for equivalence testing of salbutamol efficacy with the MSLI and ACI. It is further noted that we did not investigate FPDs lower than 9.2 μg in vivo. It is not known whether a lower FPD will result in equal efficacy as the FPD of 9.2 μg. When taking into account the standard deviation of this FPD, the test and reference product should therefore both have FPDs of at least 10 μg in order to be able to decide for equivalence based on in vitro deposition data.

In the same way the upper limit could be calculated by assigning the FINE as the test product and COARSE as the reference product. The lower limit of the 95% one-sided CI of the ratio is 3.02 for the ACI and 2.26 for the MSLI. This would result in an upper limit for the 90% CI for the ratio of 2.20. However, we only investigated efficacy. It is not known whether a test product with a FPD of 30.0 μg has the same safety as a reference product with a FPD of 9.2 μg. The earlier proposed upper limit of 1.20 should there-

fore be maintained. If this upper limit is exceeded, comparative in vivo safety studies must be performed in order to substantiate equivalence, for example by measurement of the systemic bioavailability.

Several studies have shown that the FPD is correlated with the amount of drug deposited in the lung (Seale and Harrison, 1998; Leach, 1998; Laube et al., 1998). The results of our study show that there is no difference in clinical efficacy of salbutamol preparations with a low or high FPD, within the doses currently used in practice. This is supported by data from Melchor et al., 1993, who showed that differences in lung deposition of radiolabelled salbutamol did not result in differences in bronchodilatation. Our results also confirm the study of Fishwick et al. (2001), which revealed that the dose–response curves of 50+50+100+200 μg salbutamol did not differ from 100+100+200+400 μg. Since increasing the dose is equivalent to increasing the FPD, these findings also indicate that there is no difference in bronchodilator effect between high and low amounts of salbutamol deposited in the lung. Our study and the results of Fishwick et al. (2001), reveal that already with a low starting dose of salbutamol a maximum amount of β_2-receptors in the 'reachable area' is activated. Though not within the scope of our study, this suggests that for salbutamol lower doses than those currently recommended may be used.

5. Conclusion

In conclusion, in vitro equivalence testing is allowed in case there are no relevant differences between delivered dose, inhalation device and excipients (Weda et al., 2000). The MSLI and ACI are suitable for such an in vitro equivalence test. For salbutamol dry powder inhalers the earlier proposed in vitro equivalence limits for the 90% CI of the FPD ratio of the test and reference product can be widened to 0.50–1.20 provided that the FPDs (particles <6 μm) of the test product and reference product are both at least 10 μg.

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