

# Considerations for the Development and Practice of Cascade Impaction Testing, Including a Mass Balance Failure Investigation Tree

THE PQRI PARTICLE SIZE DISTRIBUTION MASS BALANCE WORKING GROUP:

DAVE CHRISTOPHER, M.S.,<sup>1</sup> PAUL CURRY, Ph.D.,<sup>2</sup> BILL DOUB, Ph.D.,<sup>3</sup>  
KENNETH FURNKRANZ, Ph.D.,<sup>4</sup> MARTIN LAVERY, B.Sc.,<sup>5</sup> KARL LIN, Ph.D.,<sup>4</sup>  
SVETLANA LYAPUSTINA, Ph.D.,<sup>6</sup> JOLYON MITCHELL, Ph.D.,<sup>7</sup> BRIAN ROGERS, Ph.D.,<sup>4</sup>  
HELEN STRICKLAND, M.S.,<sup>8</sup> TERRENCE TOUGAS, Ph.D.,<sup>2</sup> YI TSONG, Ph.D.,<sup>4</sup>  
and BRUCE WYKA, M.S.<sup>1</sup>

## INTRODUCTION

**A**PSD MEASUREMENTS OF OINDP are performed in order to characterize the size distribution of particles emitted from the OINDP device. APSD measurements are performed during drug product development for characterization studies, clinical release, and stability studies. In addition, some type of APSD measurement is usually required for release of the final commercial product as part of a comprehensive program to ensure quality of marketed batches. These measurements are made using a CI/MSLI that fractionates the incoming aerosol into several classes with well-defined limits in terms of aerodynamic particle size.

It is normal to collect data from a CI/MSLI measurement initially as mass of API collected on each of the components of the apparatus (e.g., induction port, pre-separator (if used), stages of the CI/MSLI, and back-up filter). After determining the mass of API on each component of the appa-

ratus (normally via HPLC with spectrophotometric detection or via direct spectrophotometric analysis), the arithmetic sum of the obtained individual values is calculated, expressed as % of target delivery per actuation, and is referred to as the mass balance (MB). MB is useful in determining whether an expected mass of drug has been captured by the impactor to provide a reliable measurement of the APSD, but by itself does not ensure that the APSD results are valid. MB should therefore not be used alone as a system suitability test when assessing APSD.

The PQRI Particle Size Distribution Mass Balance Working Group was formed in late 2001 to examine several issues concerning the MB specification recommendations in the following FDA Guidances for Industry:

- (i) Draft Guidance for Industry—Metered Dose Inhaler (MDI) and Dry Powder Inhaler (DPI) Drug Products Chemistry, Manufacturing, and Controls Documentation<sup>1</sup>

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<sup>1</sup>Schering-Plough Research Institute, Kenilworth, NJ.

<sup>2</sup>Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT.

<sup>3</sup>U.S. FDA, St. Louis, MO.

<sup>4</sup>U.S. FDA, Rockville, MD.

<sup>5</sup>Aventis, Pharma Ltd., Holmes Chapel, UK.

<sup>6</sup>Gardner, Carton and Douglas, Washington, DC.

<sup>7</sup>Trudell Medical International, London, Ontario, Canada.

<sup>8</sup>GlaxoSmithKline, Inc., Research Triangle Park, NC.

Mention of any particular apparatus in this document does not imply endorsement of such apparatus by the U.S. Food and Drug Administration (FDA) or the Product Quality Research Institute (PQRI).

This document provides a state-of-the-art review of the potential causes of CI/MSLI measurement failure, but does not replace the need, as part of the product development, to identify, understand, and correct product-related factors that might lead to measurement failures.

- (ii) Draft Guidance for Industry—Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products Chemistry, Manufacturing, and Controls Documentation<sup>2</sup>

A Work Plan<sup>3</sup> was developed during the first quarter of 2002, in which it was recognized that several difficulties, including the lack of guidance on diagnosing problems with CI measurements, have inhibited the development of a cross-industry approach to the use of the MB from a CI test. To meet the need for consistent guidance on CI measurements, the Working Group undertook to:

- (a) Identify points to consider in the development of cascade impactor methodology
- (b) Address points to consider during set-up and operation of a cascade impactor for testing
- (c) Develop a flow diagram, which should be followed for investigating MB failures

MB failures are defined here as MB results that fall outside acceptance criteria.<sup>4</sup> It is outside the scope of this paper to propose or evaluate specific acceptance criteria for MB.<sup>5</sup> However, it is within the scope and is one of the objectives of this paper, to identify the appropriate course of action once a failing MB result has been obtained. Another objective of this paper is to outline considerations that need to be taken into account during the development and performance of a CI test in order to minimize the occurrence of MB and APSD failures.

MB failures could arise from inadequate method development and validation. This paper

presents points to consider in the development of a robust<sup>6</sup> CI/MSLI method. However, even robust methods do not preclude errors during CI/MSLI set-up, operation, and day-to-day use, which could lead to APSD or MB failures. Discussion of such errors is included in this paper. Finally, this paper outlines the steps recommended for investigating an MB failure.

This document provides the starting point, in which the Working Group members have identified and analyzed causes of CI/MSLI test failures based on their knowledge and experience. As a next step of this process, the Working Group will conduct a confidential survey of CI/MSLI users to obtain further information about such causes. The present document may consequently be revised based on the survey results. In particular, the hierarchy of actions for investigating MB failures will be linked to the probability of each cause as determined by the statistical analysis of the survey results.

## DEVELOPMENT OF CI/MSLI METHODOLOGY

MB failures could arise from inadequate method development and validation. This section discusses the factors that should be considered in the development of a method using a CI or MSLI. These are generally applicable regardless of the method employed for quantitation, e.g., HPLC or spectrophotometry. A summary of these factors and their potential effect on APSD and MB are given in Table 1. Each factor is discussed in detail in the subsections that follow.

TABLE 1. FACTORS THAT SHOULD BE CONSIDERED IN CI/MSLI METHOD DEVELOPMENT

<i>Method development: Factor</i>	<i>Potentially affects</i>	
	<i>MB?</i>	<i>APSD?</i>
A. Solvent	Yes	Yes
B. Quantitation lower limit	Yes	Yes
C. Use of collection surface coating	Yes	Yes
D. Recovery techniques	Yes	Yes
E. Use of a pre-separator	No, except for carrier based DPIs	Yes
F. Cleaning procedure	Yes	Yes
G. Electrostatic charge	Yes	Yes
H. Environmental factors (barometric pressure, temperature, humidity)	Yes	Yes
I. Use of a back-up filter	Yes	Yes

### *API recovery solvent*

The ability of the chosen solvent to effect dissolution of the drug and thus recovery of API from the impactor collection surfaces and accessories is critical to the robustness of the method. The choice of solvent can also influence chromatography and potentially impact API quantitation. Hence, both MB and APSD could potentially be affected. Therefore, it is critical to consider and choose a solvent appropriate for a particular drug formulation.

### *Quantitation lower limit*

The quantitation lower limit should be determined with the chosen solvent that will be used to recover the API from the collection surfaces and accessories. This limit should guide the decision about the number of actuations required in the test so that an adequate amount of drug can be deposited on the collection surfaces. The effect of any coating agent on the quantitation limit should be assessed if the collection surfaces are coated with an agent to retard or prohibit particle bounce and to prevent re-entrainment of the particles within the airflow within the impactor. The validity of the MB or APSD or both may be compromised if either the quantitation limit or the number of actuation per determination is inadequate.

### *Use of collection surface coating agents*

The need for a collection surface coating agent to prohibit particle bounce and prevent re-entrainment of the particles in the airflow within the impactor to lower stages should be assessed early in the development before extensive intermediate precision studies are undertaken.

For aerosols comprising liquid droplets, collection surface coating agents may or may not be used, but their use may need to be evaluated during method development.

Collection surface coating agents are typically used to enhance deposition and adherence of the dry powder particles on the collection surfaces when testing DPIs.

There have been many published studies for PMDIs recommending coating of the impactor collection surfaces,<sup>7-13</sup> despite the fact that this class of OINDP typically contains co-solvents

and/or surfactants, which may aid in the adherence of the particles to the collection surfaces.

If collection surface coating agents are used, the amount, application technique, and coating uniformity should be assessed during method development, as these factors could affect deposition and distribution patterns as well as stage wall losses, thus potentially affecting both the MB and APSD.

Particular attention should be paid to the choice of collection surface coating agents, since the coating agent could introduce either a positive or negative bias in the recovery of the API.

If the agent interferes with the detection of the API, the apparent mass recovered could be enhanced. Conversely, if the API is retained in the coating agent matrix upon addition of the recovery solvent, the apparent mass will be less than the true value.

In addition, there could be a positive bias if the coating agent co-elutes with the API in HPLC and possesses some UV absorption at the detection wavelength. However, there also exists the possibility of a negative bias, even if the API is fully recovered from the collection surfaces, since the coating agent could also reduce spectrophotometric sensitivity to the API.

### *Recovery techniques*

The procedure for recovering the drug from the apparatus and accessories should be optimized so that a robust method for recovering the API is developed. A method must be established that assures quantitative recovery of API from the collection surfaces, especially where solubility in the recovery solvent is poor. Special care is required when collection surface coating agents are used.

Other aspects that should be investigated include the following:

1. Volume of Recovery Solvent: This quantity should be adequate for quantitative recovery yet not be excessive to the point at which analytical sensitivity is jeopardized.
2. Container Type: If a container (e.g., plastic bag, beaker, dish) is used to contain the recovered API, the lack of significant losses of the API to the walls by sorptive processes as well as the absence of contaminants should be confirmed during method development.

3. Recovery Technique: Straightforward immersion-rinsing is likely to be effective for highly soluble species. However, mechanical/ultrasonic agitation followed by rinsing may be necessary when the API is less soluble.
4. Contact Time: The time required to achieve complete dissolution of the API in the recovery solvent should be established.
5. Other Considerations: Thermal and photo-stability of the API in the recovery matrix may also need to be investigated and processes put in place to ensure that degradation is minimized.

#### *Use of a pre-separator*

The decision whether or not a pre-separator is appropriate should be made during method development. The omission of the pre-separator when required will have a large impact on APSD (particularly for carrier-based DPIs), while leaving MB unaffected, since the mass of API that might have been retained as large particles by the pre-separator will instead be relocated within the impactor itself.

#### *Cleaning procedure*

Some losses of API, chiefly to the back faces of the stage nozzles and in inter-stage passageways, are inevitable with CIs and MSLIs. Ideally, such losses should be less than 5% of the target delivery per actuation, based on the criterion given in <601> of the U.S. Pharmacopeia.<sup>14</sup> A procedure is normally developed for each formulation that ensures that the internal surfaces of the CI or MSLI are cleaned after a specified number of determinations, defined such that the impact on MB from internal deposition of API is less than 5% of the target delivery per actuation. If cleaning is not done, apart from the possibility for increased losses reducing MB, the potential exists for nozzle clogging, which will affect APSD. Furthermore, the risk increases that accumulated deposits from previous measurements could become dislodged and collected, thereby increasing MB.

The MSLI is less vulnerable to internal losses at the nozzles, since the presence of liquid close to the nozzles of each stage effectively will prevent the accumulation of deposits at these locations.

#### *Electrostatic charge*

There is evidence that certain CIs with electrically insulated surfaces may be vulnerable to unpredictable deposition behavior.<sup>15</sup> Surface charge accumulation may influence APSD and possibly MB, through wall losses. Surface charge effects are formulation- and impactor-specific, and may depend on the ambient humidity. Problems posed by electrostatic charge may be difficult to solve. However, they can be avoided by using an impactor having electrically conductive surfaces, but good electrical conductivity may be difficult to achieve with collection surface coatings normally required for DPI assessments. These effects should therefore be considered in method development for each particular product.

#### *Environmental conditions*

Other issues to be considered in the development of a CI or MSLI method are the effects of barometric pressure, ambient temperature, and humidity. The CI/MSLI needs to be operated at its specified inlet volumetric flow rate. Through the ideal gas law, barometric pressure and temperature influence the volumetric flow rate of gas (air) passing through the particle size analyzer.<sup>16</sup> The relative humidity can influence mass-based flowmeters due to changes in the density of the air. Such biases may not be properly accounted for with some types of flowmeters. Consideration may be needed to account for the effects of these factors on the volumetric flow rate depending upon the degree to which these factors vary from standard conditions and the ability to accurately account for these differences. Errors associated with flow rate bias caused by non-standard environmental conditions will influence APSD but are less likely to influence MB.

#### *Back-up filter*

It may be necessary to consider the use of a back-up filter in product development when testing with impactors where the final collection component is not a filter stage, such as in the NGI. This precaution is particularly important for certain solution pMDI formulations that contain a significant proportion of the API in sub-micron sized particles. It is important that the recovery from the filter be investigated. Note that the micro-orifice collector of the NGI can be replaced

TABLE 2. FACTORS THAT SHOULD BE CONSIDERED IN CI/MSLI DAY-TO-DAY USE

Routine CI/MSLI set-up and operation: Factor	Potentially affects	
	MB?	APSD?
A. Locating collection surfaces	Yes	Yes
B. Accounting for collection surfaces and final filter	Yes	Yes
C. Assertion of stage order	No	Yes
D. Air leakage into CI/MSLI	Yes, unless wall losses are accounted for	Yes
E. Poor seal and orientation between induction port/pre-separator/CI	No	No, unless the leak is massive
F. Improper alignment between inhaler mouthpiece and induction port	No	Yes
G. Inadequate liquid volume or liquid missing from liquid-based collection surfaces	Yes, due to analytical procedure differences	Yes
H. CI/MSLI flow rate	No	Yes
I. Timer operation of two-way solenoid valve for DPI testing and MDIs with integrated spacers	Yes	Yes
J. Cleaning of stage nozzles	Yes	Yes
K. Worn/corroded stage nozzles	No	Yes
L. Insufficient or excessive number of inhaler actuations	Yes	Yes
M. Improper sample recovery	Yes	Yes
N. Electrostatic effects	Yes	Yes

with an internal filter for products in this category.

**CI/MSLI SET-UP AND OPERATION IN DAY-TO-DAY USE**

Proper set-up and operation of a CI/MSLI is critical to the APSD measurement. For example, test failures could arise if an impactor is not properly assembled or checked for proper airflow immediately prior to a collection run. Potential causes of a CI/MSLI test failure and their impact on MB and APSD are summarized in Table 2. Each cause of test failure is then described in more detail below.

*Collection surfaces*

Errors associated with the location of collection surfaces are largely restricted to CIs with collection plates, such as the ACI. For example, it is possible to incorrectly locate the plate on its support pegs or to orient it in the incorrect direction (i.e., curved lip up when used without a collec-

tion filter substrate). Collection plates can be bent or otherwise distorted if they are mishandled and should therefore always be visually inspected for flatness before each use.

In the case of impactors using collection cups, such as the MMI series and the NGI, as well as inspecting for flatness of the cup bottom, it is important to check that each cup is properly locked or located into position before use. For the MSLL, a horizontal, concentric and crack-free collection plate rising above the holder is important.<sup>17</sup> Collection surfaces that are visibly distorted or damaged should not be used.

In all these cases, both MB and APSD may be affected, since there may be increased inter-stage deposition that will generally occur adjacent to the misaligned or damaged collection surface(s).

*Accounting of collection surfaces and final filter*

An accounting of all collection surfaces should be made before an APSD measurement. Occurrences of missing stages are largely restricted to CIs with collection plates. For MMI-type CIs, it is readily noticeable from the external CI appear-

ance whether all cups are present. In the case of the NGI, a missing collection cup would result in no flow through the impactor inlet.

The after-filter may be missing from either type of impactor without its absence being evident, or the filter itself may be improperly placed or damaged during placement resulting in air bypassing the filter. The APSD may be affected if any of the collection surfaces or the final filter is missing, damaged, or improperly placed. In addition, MB may be affected, as a result of material loss due to a damaged, improperly located, or missing filter, or through increased inter-stage losses in the case of a missing stage.

#### *Assertion of stage order*

It may be possible to assemble a CI without being aware from its external appearance that the stage order is incorrect. This cause is most likely with CIs that comprise stacks of stages, such as the ACI. It cannot arise in normal use with MMI type impactors or the NGI, in which the stage nozzles are fixed into a single body. However, if the nozzles are removed for cleaning, there is a possibility of incorrect re-insertion. MB may not be affected, although the APSD may be noticeably influenced, depending on where the mis-ordering has taken place. The results from this type of error are therefore generally self-evident from the appearance of the APSD.

#### *Air leakage into apparatus*

Air leakage into the CI/MSLI can arise from incorrectly located or defective seals. The problem is particularly prevalent with the standard O-rings used with ACIs, which are prone to crack with repeated use and exposure to solvents. Defective seals are most significant when they occur at stages closest to the impactor exit, where the pressure inside the stage is at its lowest with respect to the surrounding atmosphere. Since air leaks will be into the CI, MB will likely be unaffected. However, APSD will likely change, depending on the magnitude and location of the leakage. Additionally, an increase in inter-stage deposition could occur, which could affect MB if such deposition is not included in the analysis.

#### *Poor seal and orientation between induction port/pre-separator/CI or MSLI*

Impaired sealing at the connections between the components upstream of the CI/MSLI will

not affect MB, but could influence APSD, depending on the magnitude of the leakage. However, because the pressure differential between the CI/MSLI entry and the ambient atmosphere is relatively small, such leakage would have to be large to have a discernable effect on APSD for inhalers other than high-resistance DPIs.

#### *Improper alignment between inhaler mouthpiece and induction port*

The actuator mouthpiece of the inhaler (including any add-on device such as a spacer) should be correctly oriented to align on axis with the entry to the induction port. Misalignment may result in increased inertial deposition within the induction port, and resulting loss of material that would otherwise have reached the pre-separator or entered the CI (if no pre-separator is present) or MSLI. MB is less likely to be affected than APSD. If APSD is changed, it would be shifted to smaller sizes, as the largest particles are most prone to inertial deposition within the induction port.

#### *Inappropriate liquid volume or liquid missing from liquid-based collection surfaces (e.g., MSLI or NGI pre-separator)*

If too much liquid is present in a given stage, the MSLI may function, but there is an increased risk of splash-over from that stage to the next. In the more likely condition that insufficient liquid is present, the impinger will also function, but particles may not be collected efficiently. In both instances, MB will be unaffected, but APSD will likely be biased towards smaller sizes due to increased material transfer further into the impinger.

If liquid is missing from the pre-separator of an ACI or NGI, the APSD will also be shifted to smaller sizes, but the MB may not be affected unless the API that should have been collected in this component is lost to recovery by depositing on inter-stage surfaces.

#### *Setting of CI/MSLI flow rate and leakage check*

The correct volumetric flow rate at the entrance to the induction port should be established before every APSD measurement. However, this measurement is not easy to make accurately. This is especially true for DPI testing, where it is necessary first to establish a flow with the DPI attached

to the induction port such that a 4-kPa pressure drop is achieved across the inhaler, while maintaining critical flow in the flow regulating valve, and then replace the DPI by a flowmeter and establish the volumetric flow rate. Guidance has recently been provided in European<sup>18</sup> and U.S. Pharmacopeias<sup>19</sup> for methods of setting and measuring flow rates during DPI testing.<sup>20</sup>

A suitable-sized bubble flowmeter or dry gas meter can be attached to the induction port entry to establish volumetric flow rate for the testing of other inhaler types, since as a general rule the resistance to flow of these flowmeters is minimal.

Leakage checking is directly related to the setting of inlet flow rate because air ingress other than via the intended route through the induction port will reduce the actual flow rate at entry point. A leakage test should therefore always be made immediately before every APSD measurement. Before undertaking this test, however, it is important that the mechanical integrity of all components in the flow path beyond the impactor (e.g., regulation valve, critical orifice and manometer, if used, as well as any flowmeters and interconnecting tubing) is sound. Procedures should therefore be in place for regular inspection of the entire flow pathway for each CI/MSLI set-up.

One way to check for leakage is to close the entry to the induction port, draw a slight vacuum within the impactor or impinger, isolate the apparatus from the vacuum source and observe the subsequent pressure rise with a manometer located at the inlet. Although standards for such a test are not currently in place, the User's Guide for the NGI specifies a vacuum of 2.5 kPa with a pressure rise of  $<100 \text{ Pa s}^{-1}$  for leak-testing this apparatus.<sup>21</sup> It is important not to draw too high a vacuum for this test, as otherwise imperfect seals may compact and function properly during the leak test, but subsequently fail once the vacuum is released to enable the APSD measurement to take place.

An alternative method is to use calibrated flow meters located both upstream and downstream of the impactor to compare flow rates. A decrease in the flow rate upstream compared with the nominal (assumed to be true) flow rate measured downstream is indicative of a leak somewhere in the impactor or impinger system. However, expansion of the volumetric flow at the reduced pressure within the apparatus will need to be considered, so that these measurements may be more

reliably performed using calibrated mass flowmeters.

If leakages are not corrected, the APSD will be biased, but MB is less likely to be affected, since the mass of API sampled will be retained within the system. A decreased upstream flow rate relative to the nominal value will shift the measured APSD to smaller sizes, and the converse is true.

#### *Timer operation of two-way solenoid valve for testing DPIs or MDIs with integrated spacers*

Proper operation (including complete time curve) of a two-way solenoid valve should be determined through regular performance check. The mass of active component delivered from the DPI or MDI with integrated spacer may depend on the duration for which sampling takes place. Hence, MB may be increased if the timer operates for longer than the required duration, and decreased if the sampling time is shorter. The APSD measurement may be biased, depending on the flow dynamics associated with an incorrect sample time.

#### *Cleaning of stage nozzles*

Excessive accumulation of deposits in stage nozzles could affect APSD determinations. In general, all CIs have small inter-stage losses ( $<5\%$  per USP  $<601>$ ), and the most likely location for such deposits is on the back face of the nozzles. If the CI is not cleaned periodically (this frequency is formulation-specific), it is possible that debris may break loose and be collected on a stage downstream. Under these circumstances, both MB and APSD may be affected. If nozzles are routinely cleaned as part of the analysis, this should not be a problem.

#### *Worn/corroded/clogged stage nozzles*

Impactors manufactured from certain metals, particularly aluminum, can have a tendency for the nozzles to wear and/or corrode with repeated exposure to formulation and recovery solvents. Stage mensuration, carried out on a regular basis will detect such behavior. However, if wear/corrosion/clogging takes place, APSD will be biased even though MB is unlikely to be affected. The APSD bias will occur in the direction of finer particle sizes in the case of worn or corroded nozzles, when the jet diameters affecting the total cross sectional area of the stage increase from

their design size. Conversely, the APSD will be biased toward coarser sizes when corrosion or clogging of individual nozzles decreases the total jet cross-sectional area.

Although adequate cleaning procedures for the CI should have been established during product development, clogged nozzles associated with a given stage or stages may conceivably occur in day-to-day product testing, resulting in a change in the APSD by a reduction in total jet cross-sectional area. Normally, the stages upstream of the clog would not be affected by its presence unless the clogging was so severe that the design flow rate through the CI was not attained. The stage with the clog would typically be associated with a higher than expected amount of API, and the subsequent stages would have lower than expected API recoveries. The solution is to inspect the nozzles of the suspected stages by microscopy.

#### *Insufficient/excessive number of inhaler actuations*

For most formulations, multiple actuations are required to achieve the required analytical sensitivity for the APSD measurement. If the operator delivers less than the specified number of actuations, both the mass per stage and MB will be reduced, but the shape of the APSD will be unaffected, since the size-fractionating capability of the CI/MSLI will not be impaired. If an excessive number of actuations is delivered, MB and mass per stage will be increased. The shape of APSD may also be affected, depending on the nature of the formulation (e.g., unit dose strength, excipient mass/dose, particle properties). For example, in the case of excessive number of actuations, APSD is likely to shift to smaller sizes because of stage overload leading to the increased particle "blow-off" to stages further within the CI.

#### *Improper sample recovery*

This paper earlier listed some of the factors to be addressed when developing a proper recovery technique for a particular formulation. It is expected that inadequate recovery procedure (e.g., for a poorly soluble active species) will be identified and amended during such method development. However, errors in recovery technique may arise during routine use by a particular CI operator. Depending on whether or not the problem is associated with one stage or through-

out the CI, the shape of the APSD may or may not be affected, but the MB and mass per stage will always be lower than expected.

#### *Electrostatic effects*

Although an improbable source of error with impactors manufactured predominantly from metal components, electrostatic charge acquisition on non-conductive parts (e.g., inhaler actuators, add-on devices such as spacers) may result in non-ideal aerosol behavior during the measurement process. Such effects are difficult to quantify due to the unpredictable nature of electrostatic charging processes and their dependence upon ambient conditions, particularly relative humidity.

Electrostatic charging can affect both MB and APSD, and should therefore be minimized. Precautions that can be taken include the grounding of staff making measurements and the use of conductive clothing including gloves. Manufacturers' protocols for pre-treatment of add-on devices to minimize electrostatic charge (e.g., washing) should also be followed where appropriate to do so.

### **CI/MSLI MB FAILURE ANALYSIS**

As defined in the Introduction, MB results are considered failures if they fall outside acceptance criteria. Therefore, for a failure analysis to be meaningful, it is important that such acceptance criteria be established based on method capability and other factors determined in method development and validation. If pre-determined acceptance criteria (e.g., such as those recommended in the FDA draft Guidances for OINDP<sup>22</sup>) are used, they need to be put in perspective with respect to method capability.

#### *Accounting for method variability in setting MB or APSD acceptance criteria*

Depending on the specific sampling plan and magnitudes of the product and method variabilities, MB or APSD failures could occur simply because the CI/MSLI test method variability encompasses or exceeds the entire range of the pre-determined acceptance criteria. In this instance, the failure analysis is meaningless because there is insufficient method capability to meet the pre-determined criteria. After separating method

from product variability, either the method (measurement technique and sampling plan) needs to be revised to reduce variability, or the acceptance criteria need to be widened.

In order to quantify and therefore properly account for the components of variability of the overall test results, a specifically designed experiment should be considered. The relative contributions of various sources of variability—such as test method (including variability due to analyst, instrument, and day/time conditions), product, and sampling plan—may then be obtained through statistical analysis.

Implications of using pre-determined acceptance criteria, rather than criteria based on the actual method capability, may not be self-evident and should be considered specifically. For example, if the same acceptance criteria derived using multiple actuations are applied in a situation when only the minimum patient dose is used for a CI/MSLI determination, the results obtained with the smaller number of actuations representing minimum patient dose will be subject to greater variability. This increased variability will lead to random failures regardless of product quality or the proper execution of the method. Thus, because such fixed and pre-determined acceptance criteria may be inadequate, method capability should be established and taken into account when setting actual MB and APSD acceptance criteria for the OINDP product.

#### *Documenting causes of failure in case of appropriately set acceptance criteria*

The MB failure analysis presented below assumes that the acceptance criteria already account for method capability and other method- and product-specific factors determined during method development and validation.

The presented analysis is restricted to the process of measuring APSD and MB of OINDP. It excludes failures arising from causes outside the measurement process, such as improper CI/MSLI manufacture. It is assumed that CI/MSLI instrument deficiencies will be identified and addressed at Installation Qualification. In use, the CI should be periodically inspected and subjected to stage mensuration to verify that the critical dimensions (specifically the nozzle diameters) are within the manufacturer's specifications.

As in any failure analysis, failure of a CI/MSLI

MB determination should be investigated in an attempt to determine an assignable cause for the failure and decide on an appropriate action. Even with proper consideration of the factors previously discussed in the development of the method, and proper CI/MSLI set-up and operation, failures can occur. Sometimes the cause is readily apparent, and at other times, the exact nature of the cause is ambiguous or indeterminate. The difficulty lies in the complexity of the test. The intent of the following subsections is to discuss the different possible causes of failure, in the descending order of their likelihood, and to offer a logical investigation path that can be used routinely.

In case of an MB or APSD failure, established general procedures for investigating failures should be followed. Broadly speaking, a mass balance failure investigation should focus on the following four sources of error: (1) analyst, (2) environmental conditions, (3) equipment, and (4) product (Fig. 1). The details of each step are described below. The given order of investigation should be followed, as it reflects the likelihood of each error type.<sup>23</sup> These recommendations, however, are not meant to supercede individual organization-based Standard Operating Procedures for dealing with laboratory investigations and out-of-specification results.

We emphasize that the list of possible errors that follows is meant to guide the investigation required in the case of MB/APSD failure, and is included here for the purpose of completeness. We further emphasize that appropriate management system, laboratory procedures, and training must be in place so as to insure correct, consistent, and verifiable operation of the cascade impactor. To underscore that the items listed below refer to the situation of a failure investigation, rather than to a routine operation of a CI/MSLI, the text is highlighted in *italics*.

*Failure investigation of the analyst. The list of possible analyst-related errors presented in this subsection is meant to guide the investigation required in the case of failure.*

*Analyst errors can be minimized by the effective implementation of robust policies and procedures by supervisory management including comprehensive analyst training and periodic reassessment. These procedures should incorporate methods for identifying sources of errors, correcting them, and where possible, means for minimizing occurrence of similar errors in the future.*

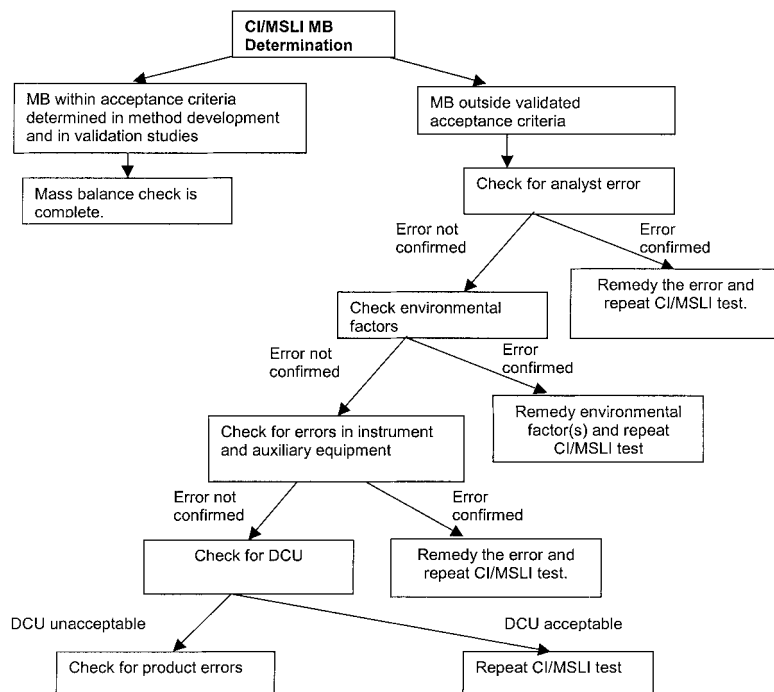


FIG. 1. A CI/MSLI MB failure investigation tree (draft).

The investigation of analyst-related errors would be facilitated if the analyst follows and initials a step-wise checklist to confirm to the extent possible the correct performance of procedural steps and use of appropriate equipment. This approach may be particularly helpful when training new analysts or introducing modifications to the method, for instance, associated with testing a new formulation. To be of use, such a checklist should be product-, apparatus- and method-specific. A checklist would help the analyst remember to carry out all the steps of a procedure and reduce omissions, and would facilitate the investigation in case of acceptance criteria failure. While the use of a checklist may not guarantee total absence of human error, it may provide some benefits and thus, the implementation of a checklist should be considered in the overall context of the quality systems of the organization.

Analyst errors are difficult to prove with certainty (which does not change the fact that they are analyst and not product errors) unless there is some physical evidence, which demonstrates that in fact, or with high probability, an error or errors have occurred. In accordance with standard laboratory procedures, after the discovery of an APSD or MB failure, an investigation will be initiated in an effort to establish an assignable cause. The following possibilities a–g (listed in no order of priority) should be explored:

- (a) **Transcription, calculation, assignment, or measurement/integration errors.** All numbers and formulae associated with, and calculations leading to the result should be checked to confirm whether any errors have been made, including sample identity. Incorrect assignment of the stage number to the sample solution being analyzed will result in reporting an incorrect APSD. It is also possible that an error in the measurement or integration of the peak or peaks of interest has been made, and thus the chromatograms, integration parameters, and any computer outputs should be checked. These types of errors can be confirmed since typically evidence is readily apparent.
- (b) **Use of incorrect analytical method.** Use of the correct analytical method should be checked and confirmed. This error can be confirmed since methods are typically identified by an unambiguous numbering or identification system. Even if the correct analytical method was used, each critical step in the procedure should be reviewed to confirm, as best as possible, whether a step was omitted or performed incorrectly. In many cases, omissions or errors can be substantiated, but situations can arise where only speculation is possible as to their presence.
- (c) **Dilution errors.** Inadvertent dilution errors in particular can result in both APSD and MB fail-

ures. Some procedures require different dilution volumes for different stages depending on the mass of API collected, and these dilution volumes will have been established during method development. However, incorrect sample dilutions may occur as a result of operator error, and generally they result in an over- or underestimation of MB as well as APSD changes. Under-dilution may result in the sensitivity of the analyte detection system becoming non-linear or in incomplete dissolution of the API. Under these circumstances, the central region of APSD may be reduced in relation to the extremes. Over-dilution of the sample may result in disproportional loss of sensitivity close to the detection limit, particularly for stages where little material is collected, which may also result in a slightly lower MB. In the latter instance, the width of the APSD may become narrower than expected, although the central portion is likely to be unaffected. In some cases, dilution errors can be proven if the analyst retains the glassware until the results have been calculated. However, given the number of measurements per analyst per day, and the established procedures by which the work throughput is managed in a pharmaceutical laboratory on a day-to-day basis, it is likely that by the time that a MB failure is discovered, apart from inspecting data entries for obvious errors, there is little that can be done to confirm positively a dilution error as the source.

- (d) **Use of incorrect solvents, reagents, or standard reference material.** The investigator should check if the correct solvents, reagents, or standard reference materials have been used and are within their assigned expiration or acceptable use period.
- (e) **Use of incorrect sample.** Use of the correct product, sample, or batch for the analysis should always be checked. This type of error can easily be confirmed unless the sample has been discarded.
- (f) **Use of incorrect equipment (e.g., glassware, HPLC column).** The investigator should check whether the correct apparatus has been used. This includes use of correct pipettes, volumetric flasks, and HPLC column, for example.
- (g) **Misapplication of technique.** Unintentional errors arising from improper application of technique could still occur, even when following the steps in a method as given in the protocol and complying with the checklist. For example, even with a robust method, occasional incomplete dissolution of the API may occur for a variety of reasons, including analyst fatigue or distraction. Such a situation will affect both the APSD and MB. This type of error can be difficult to prove.

Failure investigation of environmental factors. If the procedure requires that the test be performed under specified ambient temperature and/or humidity conditions, the accuracy of the temperature and relative humidity measurements needs to be verified. This source of error is especially important where the formulation is hygroscopic or deliquescent. Consideration of the impact of any clothing (e.g., protective gloves) should be made if the formulation delivery has been shown to be affected by electrostatics.

Failure investigation of instrument. After discussing the events and methodology with the analyst and verifying the correct environmental conditions, the investigator should focus on instrumentation. This includes the HPLC system, CI/MSLI set-up, and any other equipment used in association with the analysis. System suitability criteria for the HPLC should be rechecked.

- (a) **Calibration.** All employed ancillary instrumentation (e.g., flowmeters) should be checked to confirm that the used conditions are within the calibration interval of the instruments and that the instruments have successfully passed their latest calibrations. Even if all such equipment is within calibration and the system suitability criteria have been met at the start of making the CI/MSLI determination, the (small) possibility of subsequent equipment malfunction and/or breakdown should not be discounted.
- (b) **Critical dimensions.** The CI/MSLI itself should be checked to confirm that all critical dimensions (principally the nozzle diameters) are within the specified ranges. Stage mensuration will normally be undertaken on a routine basis as part of the operations qualification for the CI/MSLI. However, the (small) possibility that excessive wear/corrosion/plugging of the nozzles has taken place since the most recent inspection should not be discounted.
- (c) **Impactor leaks.** There is very little likelihood of a major leak into the CI if both inlet and outlet flow rates are measured before a determination, or if a specific test for leakage is employed. However, the O-rings should be inspected routinely prior to set-up for wear and cracks around the stages that show changes when compared to what has been observed in the past. Worn, cracked or torn gaskets can cause leaks into the impactor. O-ring seals should therefore be checked as part of the instrument/auxiliary equipment inspection (Fig. 1) in the event of a mass balance failure.

Failure investigation of product. If none of the earlier steps of the investigation as described above has confirmed an error and thus a product failure is suspected, an investigation of product-related issues should be conducted. Such investigation should start by reviewing the performance of the product in the Dose Content Uniformity (DCU) test, which is the appropriate metric to determine the acceptability of delivered (emitted) dose.

Additionally, wherever possible (e.g., for multi-dose products), CI/MSLI testing of additional doses from the unit in question (and where prescribed by the sampling plan, followed by testing of additional units from the batch) should be performed. In general, true product failures are repeatable.

Product failures are product-specific. Due to the many different types of inhalation products on the market and under development, and their inherent subtleties and nuances, this paper does not intend to provide a comprehensive list of product issues that could lead to MB or APSD failures. A strong understanding of how the formulation and container behave, obtained during the development of the product, is an essential prerequisite before embarking on a diagnosis of a product-related MB or APSD failure.

Finally, it should be stressed that MB failures by themselves are not automatically indicative of product failure. It should also be stressed that an acceptable MB does not necessarily indicate that the APSD results are valid, nor does it confirm the absence of analytical errors.

## IMPLICATIONS OF TREATING MB AS PRODUCT SPECIFICATION

MB/APSD may fail for reasons not related to product quality. Therefore, it is important to have an opportunity to retest MB/APSD to investigate the error. However, if MB is treated as a product specification, the regulatory rules dictate that retesting is only allowed if an assignable cause of failure is found. Unfortunately, in the CI/MSLI test, many types of non-product-related errors are difficult if not impossible to prove. Thus, treating MB as a product specification forces rejection/recall of a batch in case of an MB failure with no assignable cause, even though the product quality may be excellent. It is emphasized therefore that MB should be used only as one of the diagnostics in assessing the validity of the APSD measurement and not be treated as a product specification, which precludes re-testing in the case of an MB failure with no assignable cause.

## CONCLUSION

This paper describes the wide variety and intricate interplay of factors that should be considered in developing a robust CI/MSLI method, day-to-day operation of a CI/MSLI, and the steps to be undertaken when investigating an MB or APSD failure. The paper especially focuses on causes that may lead to MB or APSD failure.

The discussion presented in this paper demonstrates that MB by itself should not be used as a system suitability test, because even with an acceptable MB, the APSD measurement may be erroneous. For the same reasons, MB should not be used as a measure of delivered dose from the inhaler, for which a separate and more accurate test is performed. Other information about the APSD test is required in addition to MB in order to assess unambiguously the validity of APSD results. The presented discussion also suggests that failures of MB are not necessarily linked to product quality.

An MB within expected limits merely indicates that the measurement apparatus collected the expected mass of drug. Therefore, the MB can be used as one of the diagnostics in a standard procedure to assess the validity of the APSD data.

## ABBREVIATIONS

ACI	Andersen (8-stage) cascade impactor as defined by apparatus 1 or apparatus 3 in <601 > of the U.S. Pharmacopeia.
API	Active pharmaceutical ingredient.
APSD	Aerodynamic particle size distribution. APSD measurements yield the distribution of particles according to their aerodynamic diameter, which takes into account particle density and shape.
CI	Multi-stage cascade impactor (e.g., ACI, MMI or NGI).
DCU	(Delivered, or emitted) dose content uniformity.
DPI	Dry powder inhaler.
HPLC	High-performance liquid chromatography.
MB	Mass balance determined as a sum of the amounts of API collected from all stages of a CI, including the induction port and pre-separator (if used), as % of target delivery per actuation.

MSLI	Multi-stage liquid impinger (e.g., apparatus 4 in <601> of the U.S. Pharmacopeia).
MMI	Marple-Miller impactor (e.g., apparatus 2 in <601> of the U.S. Pharmacopeia).
NGI	Next generation pharmaceutical impactor.
OINDP	Orally inhaled and nasal drug products.
pMDI	Pressurized metered dose inhaler.

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Address reprint requests to:  
Jolyn P. Mitchell, Ph.D.

Trudell Medical International

725 Third Street

London, Ontario, Canada N5V 5G4

E-mail: [jmitchell@trudellmed.com](mailto:jmitchell@trudellmed.com)