ANALYTICAL QUALITY BY DESIGN TO CHARACTERIZE INHALATION PRODUCTS

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Master's Dissertation in Pharmaceutical Biotechnology, conducted under the scientific guidance of Prof. Doctor Luís Fernando Morgado Pereira Almeida, professor at Faculdade de Farmácia da Universidade de Coimbra and Master Maria Carlos Lopes, stability group leader at Hovione FarmaCiencia SA, presented to the Faculdade de Farmácia da Universidade de Coimbra.

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Resumo

O presente trabalho foi baseado no desenvolvimento de um método analítico utilizando a abordagem de *Analytical Quality by Design* (AQbD). Esta abordagem é utilizada quando existe a necessidade de se obter um conhecimento completo do método e, ao mesmo tempo, controlar os possíveis fatores que podem influenciar os resultados no trabalho de rotina. O método analítico escolhido foi o *Next Generation Impactor* (NGI) que tem a capacidade de avaliar a distribuição aerodinâmica do tamanho de partícula de um aerossol. Este método foi escolhido com o objetivo de melhorar a sua robustez e, ao mesmo tempo, criar um fluxo de trabalho representativo das etapas essenciais no seu desenvolvimento.

A parte experimental começou após a realização de uma análise de risco do método geral de NGI usando uma ferramenta chamada "*Failure Mode and Effect Analysis*". Com todas as possíveis variáveis críticas identificadas, foi desenvolvido um método de NGI e foram definidas as condições ótimas para cada variável.

As variáveis testadas foram: o número de cápsulas utilizadas em cada teste, tempo e tipo de agitação de cada componente, influência do revestimento e presença de perdas através do *interstage*. Ao longo de todos os testes, verificou-se que o balanço de massa esteve sempre dentro dos critérios definidos pela *Food and Drug Administration* exceto no teste realizado sem revestimento. As variáveis que tiveram influência considerável nos resultados obtidos estão relacionadas com a aplicação da solução de revestimento e com o tempo de agitação durante a recuperação. Testes adicionais foram realizados para avaliar a estabilidade de padrões e amostras.

No final do trabalho, concluiu-se que a abordagem AQbD no desenvolvimento do NGI foi muito útil, uma vez que permitiu avaliar quais as variáveis críticas do método com maior impacto nos resultados obtidos.

Palavras-chave: Next Generation Impactor, Analytical Quality by Design, distribuição aerodinâmica do tamanho de partícula, inalador de pó seco

Abstract

The present work was based on the development of an analytical method using the approach of Analytical Quality by Design (AQbD). This approach is used when there is a need to reach a full knowledge of the method and, at the same time, to control the possible factors that can influence the results during routine work. The analytical method chosen was the Next Generation Impactor (NGI) that has the capacity of evaluating the aerodynamic particle size distribution (aPSD) of an inhalation product. This method was chosen in order to improve its robustness and, at the same time, to create a workflow representative of the essential steps on its development.

The experimental part began after the accomplishment of a risk analysis of the general NGI method using a tool called "Failure Mode and Effect Analysis". With all the possible critical variables identified, an NGI method was developed and the optimal settings for each variable were defined.

The variables tested were: number of capsules discharged per test, time and type of agitation of each component, influence of coating and presence of interstage losses. Among all the tests, it was verified that the mass balance was always within the range recommended by Food and Drug Administration except on the test performed without coating. The variables that had a considerable influence on the results obtained were related with the application of the coating solution and with the time of agitation used during recovery step. Additional tests were performed to evaluate the stability of standards and sample solutions.

At the end of the work, it was concluded that the AQbD approach was very useful on the NGI development since it allowed to evaluate which are the method variables with higher impact on the results obtained.

Keywords: Next Generation Impactor, Analytical Quality by Design, Aerodynamic Particle Size Distribution, Dry-Powder Inhaler

List of abbreviations

ATP - Analytical Target Profile

AQbD - Analytical Quality by Design

API – Active Pharmaceutical Ingredient

APSD – Aerodynamic Particle Size Distribution

CI – Cascade Impactor

CITDAS – Copley Inhaler Testing Data Analysis Software

COPD – Chronic Obstructive Pulmonary Disease

CQA - Critical Quality Attribute

DPI – Dry Powder Inhaler

DoE - Design of Experiments

DUSA – Dosage Unit Sampling Apparatus

FDA – Food and Drug Administration

FPD – Fine Particle Dose

FPF – Fine Particle Fraction

FMEA – Failure and Mode Effect Analysis

GSD – Geometric Standard Deviation

HPLC – High Performance Liquid Chromatography

ICH - International Conference on Harmonization

IP – Induction Port

LC – Label Claim

LOQ – Limit of Quantification

MMAD – Mass Median Aerodynamic Diameter

MOC – Micro-Orifice Collector

MPA – Mouthpiece adaptor

NGI – Next Generation Impactor

OIDP – Orally-Inhaled Drug Product

pMDI – pressurized Metered Dose Inhalers

PS – Pre-Separator

Q – Flow rate

QbD - Quality by Design

QC – Quality control

RPN – Risk Priority Number

RSD – Related Standard Deviation

RT – Retention Time

SDS – Sodium Dodecyl Sulfate

SSSA – Stock Standard Solution A

SSSB - Stock Standard Solution A

USP – United States Pharmacopoeia

WSA – Working Standard A

WSB – Working Standard B

μm – Micrometer

ΔP – Pressure Drop

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Chapter I – General Introduction

Pulmonary drug delivery

Obstructive airway diseases, such as Bronchial Asthma and Chronic Obstructive Pulmonary Disease (COPD), are commonly treated with drugs which are delivered by inhalation. These drugs can target the lungs directly and because of the low doses combined with a quick onset of action they have a better therapeutic index and less side effects (1)(2).

In the last two decades, a remarkable increase of the scientific interest in the technology for pulmonary delivery was observed due to the acknowledgement that the lungs can be used as a portal for systemic drug delivery. Pulmonary delivery is attractive as a route for systemic administration due to fast absorption by the massive surface area of the alveolar region, the abundant vasculature and thin air–blood barrier, and the avoidance of first pass metabolism (2). However, the effectiveness of this kind of therapy is largely dependent on the amount of drug that will reach the intended site of deposition.

The deposition pattern is determined mostly by the formulation and the delivery device, but the patients have also a decisive role. Figure I summarizes the various parameters that can affect lung deposition (3)(4).

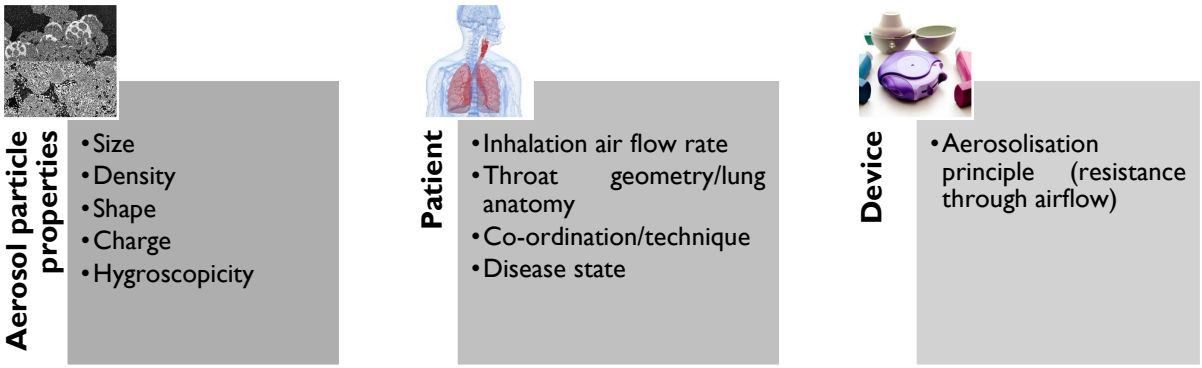


Figure I: Parameters that may affect lung deposition (3)(4).

Physiology of the airways

Anatomically, the respiratory tract can be divided in two sections, as shown in figure 2 (5), the upper respiratory system, which includes the nose, pharynx and associated structures; and the lower respiratory system, which includes the larynx, trachea, bronchi and lungs. Functionally, the respiratory tract can also be divided into two parts (6):

- Conducting zone - series of interconnected cavities and passageways which serve to prepare ambient air for respiration by warming and humidifying the incoming air, as well as filtering the air of foreign particles and pathogens;
- Respiratory zone - represents the tissues within the lungs where gas exchange between air and blood occurs (6).

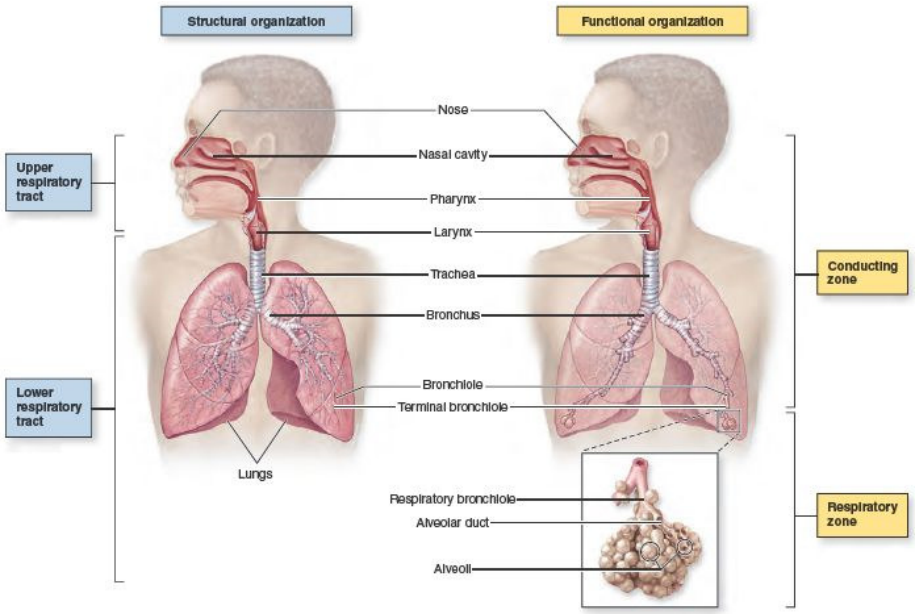


Figure 2: Respiratory tract divided by structural and functional organization (5).

The basic structure of the respiratory tract can be considered as a series of dichotomous branches. Each time the bronchial tree divides, it is referred to as a new generation. There are 23 generations of airways (see figure 3) and gas exchange occurs in the last 8.

Airway branching causes a reduction in the airflow velocity, so in the alveoli the air velocity is near zero to facilitate respiration by passive diffusion of dissolved gases. Also with the increasing number of bifurcations, the total cross-sectional area increases, so that the overall surface area of the alveolar region is very large to facilitate the absorption of systemic drugs (6).

Mechanism of particle deposition in respiratory tract

Particles deposit in the airways by three major modes of deposition mechanisms (see figure 3 (7)):

• Inertial impaction: mainly influences the deposition of larger particles that tend to collide with the airway walls in the upper airway regions. This occurs due to particle

inertia because particles continue on their original course and are unable to follow the airstream when it changes direction. Particles with larger diameters, higher densities and higher velocities will result in greater impaction (6)(8)(9).

• Sedimentation: mainly with particles in the size range of 0.5–5 μm that deposit in the terminal bronchioles and alveolar regions. This is a process proportional to the period during which the particles remain in the region. If the time between the end of inspiration and the start of exhalation is extended, there is more time for sedimentation to occur. Therefore, since the airstream velocity is relatively low, particles tend to settle due to influence of gravity (6)(8)(9).

• Brownian motion: may occur with particles smaller than 0.5 μm because of the random bombardment of gas molecules that result in particle collision with the airway walls. This type of deposition is associated with the alveolar spaces where air velocity is near zero (6)(8)(9).

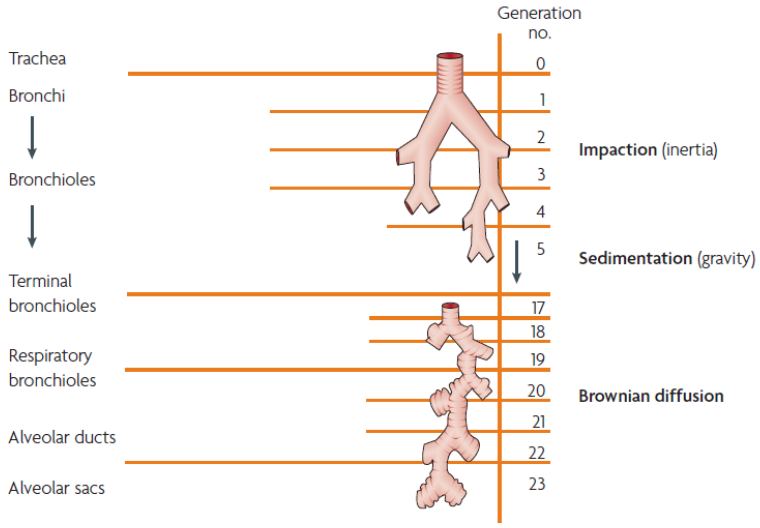


Figure 3: Factors that determine the deposition of inhaled particles (7).

Inhalation Drug Products

Devices used to deliver therapeutic agents as aerosols are divided in three major classes: nebulizers (figure 4, (10)), pressurized metered-dose inhaler (pMDI, figure 5 (11)) and dry powder inhalers (DPIs, figure 6 (12)) (2).

Figure 4: Example of nebulizer (10).

Figure 5: Example of pMDI (11).

Figure 6: Example of DPI (12).

Nebulizers

The nebulizers produce respirable droplets of the drug through the conversion of a liquid (solution or suspension) into aerosol droplets. There are three different technologies to perform the conversion of the liquid into inhalable droplets: jet, ultrasonic and mesh nebulizers. In the jet nebulizers, compressed air or oxygen are used to atomize the liquid drug into a mist. The ultrasonic nebulizers convert electrical energy to high-frequency vibrations using a transducer. These vibrations are transferred to the surface of the solution, creating a standing wave that generates aerosol. The mesh nebulizers use the ultrasonic basis to generate the droplets that then pass through a static or vibrating mesh, forming a cloud or mist of the drug (13).

These products are very user friendly, easy to use and can administer more than one product. However, they rely on very large and non-portable equipment, they need a power source and they are very expensive. It is also recognized that the drug deposition in the lungs is much lower than when compared to the other inhalation drug products (there is a high deposition on the drug in the eyes and face of the users, leading also to an increase of probable contaminations) (13).

Pressurized metered-dose inhalers

A pMDI is comprised of a solution or suspension containing the drug substance and a propellant inside a pressurized canister. When pressing the canister, the metered dose inside the canister is released in an aerosol plume by a metering valve (13).

These drug products are portable, robust, easy to handle and inexpensive and the fact that the canister is under pressure prevents possible contaminations. Nevertheless, these products have a few disadvantages as there is the need to use propellants, it is not easy to coordinate the actuation-inhalation step and they also show low deposition in the lungs (13).

Dry Powder Inhalers

DPIs are portable devices that require minimum patient coordination between breathing and actuation of the device to deliver the drug (2). Beyond that advantage, these devices (used for the current work) have the majority of the requirements

presented in the Figure 7 which contributes to the high adhesion to the treatment by the patients (1).

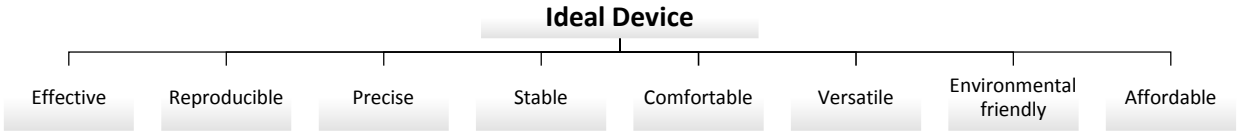


Figure 7: Characteristic of the ideal device for drug delivery.

Before inhalation, the formulation of all DPIs has no potential for lung deposition. It is the patient's inhalation that transforms the powder in a DPI into an emitted dose of particles with the appropriate characteristics for deposition in the lungs. When a patient inhales through a DPI, turbulent energy inside the device is created by the pressure drop (ΔP) that results from the interaction between the patient's inhalation flow (Q) and the internal design of the DPI which translates into a resistance to airflow.

For each inhaler there is a minimum inhalation flow required to provide efficient disaggregation of the formulation. The minimum inhalation flow, while not clearly defined for each device, is important because there is the potential for a patient to receive no dose. When a patient inhales through a DPI, disaggregation of the powder occurs almost immediately as the dose leaves the device. If the inhalation is too fast, which is possible for a DPI with a low resistance, the powder may not disaggregate before it leaves the inhaler. This situation leads to the emission of particles that are too big to be deposited in the lungs and so the dose is deposited in the oropharynx and subsequently swallowed (3).

Despite the advantages of these devices, their performance depends on the powder formulation, the design of the inhaler device and on the patient (2).

Formulation for DPIs

Traditional powder blends consist of micronized drug particles (median size 1–5 μm) blended with an inactive excipient of larger size (2). The main problem with particles of this small micron size is their high surface free energy that makes them stick to each other (via cohesive forces) or to any surface they encounter (via adhesive forces). As a result, they exhibit poor flowability and aerosolization performance and have a propensity to remain within the inhaler (14).

Drug carrier excipients added in appropriate sizes can reduce such cohesive forces, as shown in figure 8. In general, excipients improve flowability of drug particles

to facilitate filling the DPI, increase dispersion of drug particles during emission and dilute the drug to improve accurate dose delivery (achieving a good uniformity of doses) (2)(14). However, since the amount of active pharmaceutical ingredient (API) in a DPI is relatively low (0.05%–10%), a slight change in the physical properties of the carrier has a considerable effect on DPI performance (14).

Carriers used are commonly coarse particles with a size range of 50–200 mm which are designed to be swallowed after impact with the upper respiratory tract so that only fine drug particles are deposited deep in the lung. Due to the lack of toxicological data concerning the potential hazard of carriers to lung tissue, the number of carrier materials currently approved or certified safe by the Food and Drug Administration (FDA) remains limited so that most commercially available DPI formulations rely on lactose as the carrier (14).

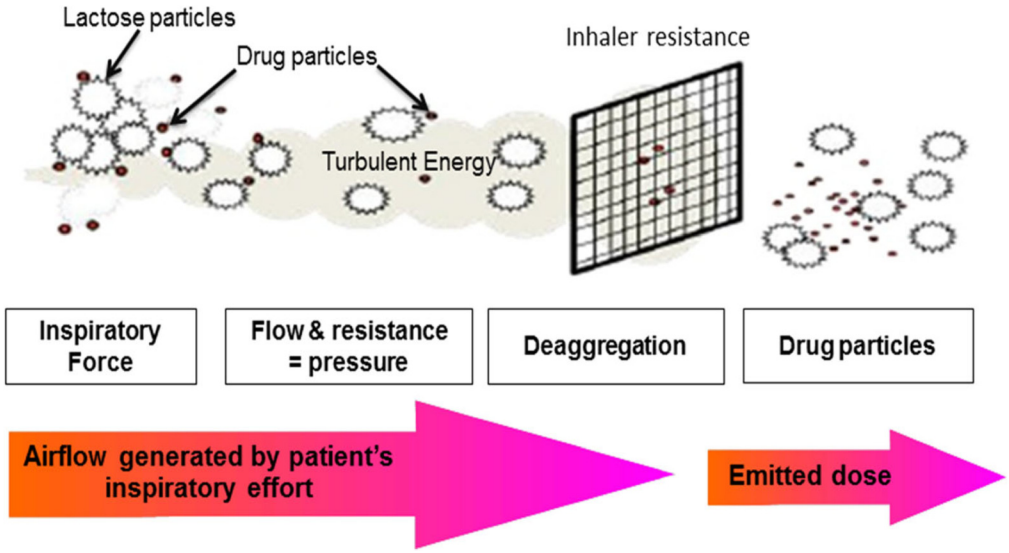


Figure 8: Schematic diagram of carrier-based formulation and dispensing powder mechanisms (14).

A specific reference should be made to drug-lactose blend which is currently the most common type of DPI formulation. A small amount of lactose fine particles (≤5-10 μm) is often incorporated to promote deaggregation in the turbulence created by inhalation. Increasing the amount of fine carrier particles in ternary interactive mixtures, up to a certain weight proportion, can improve the aerosol performance of DPI formulations. The presence of fine lactose particles can also facilitate physical disruption of the strong cohesive interaction between drug particles by decreasing the number of drug-drug contacts and increasing the separation distance between the neighboring drug particles (15).

In the present work, the formulation used consists on API X and two types of lactose. API X is used intranasally to reduce airflow obstruction related to asthma and to manage symptoms of allergic and non-allergic rhinitis.

DPI design

It is the physical design of the DPI that establishes its specific resistance to airflow. To produce a fine powder aerosol with increased delivery to the lung, a DPI characterized as having a low resistance requires an inspiratory flow of >90 L/min, a medium-resistance DPI requires 50-60 L/min, and a high-resistance DPI requires <50 L/min (16).

Critical Quality Attributes of Orally-Inhaled Drug Products

Orally-Inhaled Drug Products (OIDPs) have some Critical Quality Attributes (CQAs) that need to be controlled over the pharmaceutical development and batch release to ensure safety, quality and efficacy, namely:

• Delivered Dose (Emitted Dose) - total amount of drug emitted from the drug device and later available to the user (17)(18);

•Aerodynamic particle size distribution (aPSD) - determines the percentage of the total emitted dose that reaches the lungs or nasal mucosa during inhalation and is thus, therapeutically effective (17)(18).

The delivered dose is measured by the actuation of the test device into a sampling apparatus containing a filter (Dosage Unit Sampling Apparatus, DUSA). The dose is captured, the active drug is dissolved in solvent and an aliquot is then analysed, normally using High Pressure Liquid Chromatography (HPLC). During testing, air is drawn through the sampling apparatus to broadly simulate inhalation. The manner in which the air is drawn through the apparatus is dependent on the device under test. For DPIs, this apparatus should operate at a ΔP of 4 kPa for the duration of time to allow 4 L of air, as specified in United States Pharmacopeia (USP) <601> (17).

The aerodynamic size distribution of an aerosol cloud defines where the particles in that cloud may deposit after inhalation. In order to be effective and to deposit in the lungs, particles should be in the range of 1 to 5 μm (17)(19). Particles below 1 μm may remain entrained in the air stream being exhaled and above 5 μm particles usually impact in the oropharynx being swallowed (17)(20).

To measure particle size of inhalation products, regulators and pharmacopoeias recommend the use of cascade impactors (CIs) (17)(20).

Cascade Impactors

CIs are instruments with high precision that can separate a sample based on particle inertia without the knowledge of particle density and shape. Their widely recommendation by pharmaceutical entities is caused by three features that no other instrument have:

1. Measure aerodynamic particle size which helps to explain how particles behave in a moving air stream;

2. Measure API present in the aerosol cloud;

3. Measure the entire dose allowing complete characterization of the product in question (17).

Despite their advantages, it is important to recognize that the CI is not a lung simulator because of many features, including the geometry at the point of impact, collection surface hardness and coating, and operation at constant flow rate. In particular, collection stages in the impactor do not correspond to any specific deposition sites in the lung (21). CIs mode of operation is based on the passage of aerosolized particles through decreasing nozzle apertures onto subsequent deposition stages (15). The impaction of a particle on that stage is dependent on its aerodynamic diameter. Particles having sufficient inertia will impact on that specific stage collection plate, whilst smaller particles with insufficient inertia will remain entrained in the air stream and pass to the next stage where the process is repeated. As the nozzles get smaller, the air velocity increases, and finer particles are collected (17)(22)(23).

As long as the nozzle diameters remain within defined tolerances and there are no inherent leaks in the system, it can be seen that the cut-off diameter (the aerodynamic diameter of particles that accumulate on any given collection surface) of any stage is directly related to the volumetric flow rate of the inlet air passing through it (17).

Sometimes particles may bounce when they contact with the collection cups allowing the re-entrance in the air stream and consequently the collection on the wrong stage. Another issue that can occur is the particle deposition on another parts of the impactor instead of the collection plates (17). These occurrences may be minimized by

coating the collection cups with a suitable surface coating (e.g. glycerol or silicone oil) (17)(18)(15).

When the test is concluded, the particle mass present at each stage collection plate is recovered using a suitable solvent and then it is analyzed by HPLC to quantify the API present in the product/stage. By the quantification of the drug deposited on the various stages it is possible to calculate the Fine Particle Dose (FPD), Fine Particle Fraction (FPF), Mass Median Aerodynamic Distribution (MMAD) and Geometric Standard Deviation (GSD) (17).

The FPD is the amount of drug present in a prescribed dose that has a size capable of penetrating the lung during inhalation (e.g. ≤5 μm). The FPF is the FPD that is available to the patient, so it is expressed as a percentage of the delivered dose (17) (24).

The MMAD and GSD are what determine the site of deposition in the respiratory tract (2). The MMAD signifies the aerodynamic diameter at which half of the aerosolized drug mass lies below the stated diameter and is read from the cumulative distribution curve at the 50% point (25)(26). Taking into account the effects of aerodynamic particle size on regional lung deposition patterns, it can be seen that a MMAD ≤ 5μm of the aerosol is desirable, to facilitate predominant deposition targeted to the smaller airways. Aerosols with larger MMADs will deposit higher in the respiratory tract (6).

GSD is a measure of the variability of the particle diameters within the aerosol and is calculated from the ratio of the particle diameter at the 84.1% point on the cumulative distribution curve to the MMAD (26). The larger the GSD, the more sites that the aerosol will be deposited in the respiratory tract (15)(25). In general, aerosols with GSD <2 are desirable and ideal, since are a reflex of aerosol particles as close as possible to monodispersity, which will increase deposition at the desired site of action and consequently increase the efficacy of the treatment (2).

In pulmonary delivery of pharmaceuticals, aerodynamic size distribution is the most important parameter affecting aerosol performance. However, it should be noted that in the FDA guidance document, acceptance criteria expressed in terms of MMAD and GSD alone (representing the measures of the central tendency and spread, respectively) are not considered adequate to characterize the particle size distribution of the whole dose (15).

FDA recommends that the total mass of drug collected on all stages and accessories (mass balance) should be between 85% and 115% of label claim (LC) on a

per actuation basis (27). The Pharmacopoeias recommend some commercially available impactors for the routine testing of OINDPs, including the Next Generation Impactor (NGI) (17) (19).

Next Generator Pharmaceutical Impactor

The NGI was designed and calibrated by an industry consortium to meet the specific requirements of the industry for inhaler testing (28)(29)(21). It is a widely used equipment at inhaler research laboratories because of its flexibility, high performance, productivity and precision (22).

This CI is calibrated at a flow rate range of 30-100 L/min (with an additional calibration at 15 L/min for nebulizer applications). It is constituted by seven stages and a Micro-Orifice Collector (MOC), which allow stage efficiency, accuracy and reproducibility (19) (22).

The impactor itself comprises just three main parts (Figure 9): a removable cup tray containing the eight collection cups used to collect the samples prior to analysis; the bottom frame used to support the cup tray and the lid containing the inter-stage passageways and the seal body which holds the nozzles in place. The three parts are held together using the handle clamping mechanism (22)(30).

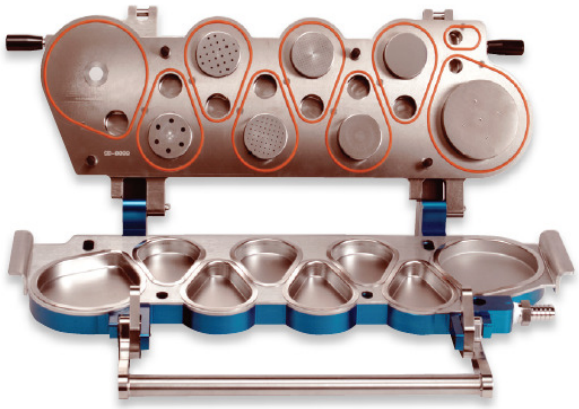


Figure 9: NGI with three main parts. (17)

This design allows minimal particle carryover and low inter-stage wall losses (<5% on any stage and 5% overall) which ensure good drug recovery (mass balance) (22)(29)(21).

There are eight nozzle pieces in the NGI, corresponding to seven size-fractionation stages and a MOC, as can be seen on figure 10 (30).

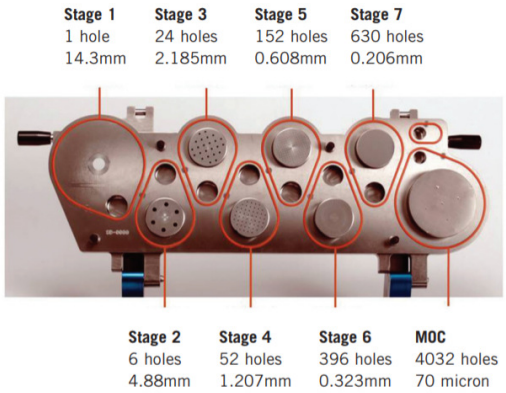


Figure 10: Layout of NGI showing size of nozzles on each stage (30).

At either end, the NGI has two larger cups, one at the beginning that minimizes large particle impaction on the stage walls (Stage 1 Cup) and another one, at the end, that avoids the need for a final filter since it allows capture and analysis of the finest particles (MOC) (22)(28)(29).

The cut-off diameters for the relevant stages at volumetric flow rates of 15, 30, 60 and 100 L/min are given below on table 1.

Table 1: Cut-off diameters of NGI stages at different flow rates (17).

Cut-off diameters at	15	30	60	100	L/min
• Stage 1	14.10	11.76	8.06	6.12	microns
• Stage 2	8.61	6.40	4.46	3.42	microns
• Stage 3	5.39	3.99	2.82	2.18	microns
• Stage 4	3.30	2.30	1.66	1.31	microns
• Stage 5	2.08	1.36	0.94	0.72	microns
• Stage 6	1.36	0.83	0.55	0.40	microns
• Stage 7	0.98	0.54	0.34	0.24	microns
• MOC	0.70	0.36	0.14	0.07	microns

The impactor is usually preceded by an entry or induction port (IP, figure 11) to ensure that the inhaler mouthpiece, which provides an airtight seal between the IP and the device, is oriented with respect to the NGI station in a fixed horizontal plane, and to ensure that the aerosol produced is sampled in a consistent manner. The IP also serves the purpose of mimicking the human oropharyngeal region (17)(22).

A preseparator (PS, figure 11) located immediately after the IP is often needed to remove particles from the aerosol exiting the IP and to avoid overloading the first stage (Stage I) (28)(31)(32).

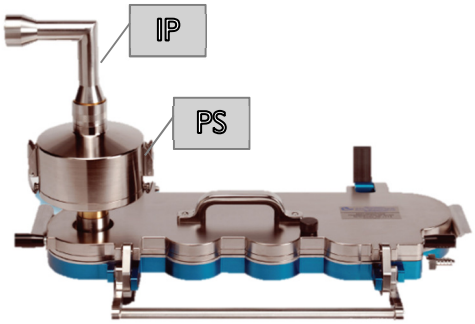


Figure 11: NGI with IP and PS (17).

To use the NGI station there are some additional equipment necessary, such as:

- Vacuum pumps (figure 12) - draw air at the designated volumetric flow rate through the system and generates sonic flow;

Figure 12: Vacuum pump (17).

- Mouthpiece Adapters (MPA) (figure 13), tubing and quick release connectors - link the various components of the system together;

Figure 13: Examples of mouthpiece adapters for different inhalers (17).

- Critical Flow Controller (figure 14)(33) - generates a standardized breath profile suitable for the routine testing of DPIs;

Figure 14: Critical Flow Controller (33).

- Flowmeter (figure 15) – measures flow rate (17).

Figure 15: Flowmeter (17).

NGI Leak Tester

The seals on CIs can deteriorate with repeated use and exposure to solvent. Since the system operates under vacuum, a leak allows entrance of air into the system causing erroneous results due to incorrect flow rates and poor aerodynamic performance.

For this reason, all CIs should be tested on a regular basis to check the integrity of the sealing system. The most common method used for leak testing is to block the

entry to the impactor inlet, generate a vacuum within the impactor using a vacuum source and then monitor any alteration in pressure using a pressure meter located within the enclosed system (17) (28).

This method is sensitive, accurate, straightforward and fast. It is ideal for verification checks during the life of the impactor or as a fast system suitability test before an impactor is used (17) (28).

Flow rate

Despite the impactors being calibrated at certain air flow rates, the measurements can be performed at any arbitrary flow rate, defined by the pressure differential over the inhaler device. For DPI performance evaluation, the pharmacopoeias (United States and European) recommendations are to set the flow rate through the impactor to generate a 4 kPa ΔP over the inhaler and a duration consistent with the withdrawal of 4 liters of air from the mouthpiece of the inhaler (19)(15)(29). This generally represents the approximate ΔP created at the inhaler entry by an adult asthmatic or COPD patient inhaling deeply (29).

To measure the ΔP created by the air drawn through an inhaler it is necessary to measure the absolute pressure downstream of the inhaler mouthpiece (see figure 14) and then this value is compared with atmospheric pressure. The flow rate from the vacuum pump is afterwards adjusted to produce the required ΔP of 4 kPa and the flow rate, Q, required to produce this ΔP is measured (17)(22).

Finally, since a stable flow is critical for good impactor measurement practice, it needs to be controlled. The importance of the flow stability resides in the dependence between the aerodynamic sizing ability of inertial impactors and the velocity of the air flow passing through each stage. That velocity is directly related to the volumetric air flow rate (17).

To validate flow rate stability, it is necessary to check if the critical flow occurs in the flow control valve. This can be confirmed by measuring the absolute pressure at a point on either side of the flow control valve (see figure 16) (17)(34). If the pressure downstream of the valve is less than half of the upstream pressure ($P3/P2 \leq 0.5$), critical flow is assured, and the flow rate can be assumed to be stable (17) (32).

If this criterion cannot be achieved, it is likely that the vacuum pump is worn or is of insufficient capacity and should be repaired or replaced (17).

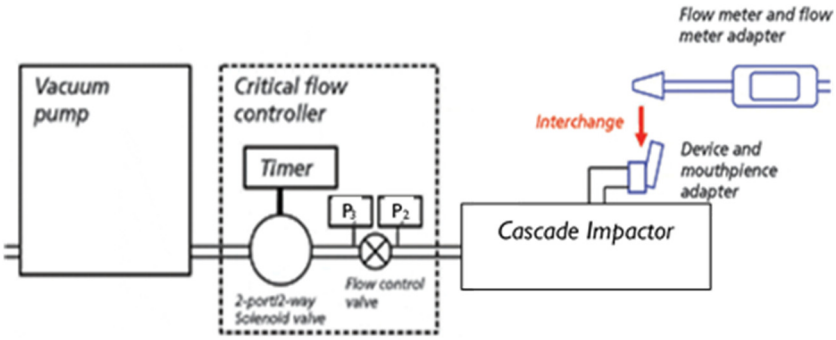


Figure 16: Adaptation of scheme that represents NGI test-setup for DPI testing (34).

API quantification by HPLC

In order to attain the final objectives of NGI method it is necessary to use a HPLC method able to quantify the API present on the different stages/components.

Liquid Chromatography is the science of separating the chemical compounds (dissolved in a solvent) that are in the sample. HPLC separation of each chemical entity from the sample mixture is based on its distinct affinity towards the adsorbent material in the column (packed with small diameter porous particles) or the mobile phase, causing various constituents to travel at different velocities and separate (35)(36).

During the run, a pump can deliver a constant mobile phase composition (isocratic) or a mixed mobile phase composition (gradient). After being injected, the analytes are pushed through the column by the mobile phase and are detected by a suitable detector that pass them as a signal to the HPLC software in the computer leading to a chromatogram (figure 17). The chromatogram allows the identification and quantification of the different analytes (shown as peaks) (37)(38)(39).

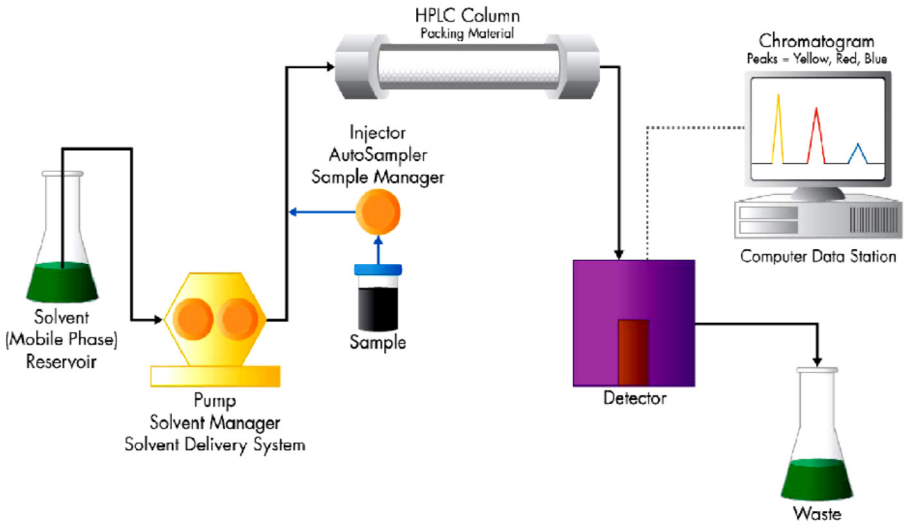


Figure 17: Workflow of an HPLC isocratic system. (37)

Among the existent separation modes used in chromatography, the most common is the reversed-phase chromatography because of its versatility. On this separation mode the column packing is non-polar and the mobile phase is polar which leads to the retainment of non-polar compounds (38).

Figure 18 represents a typical chromatographic separation of two substances, 1 and 2. t_{R1} and t_{R2} are the respective retention times (RT); h is the height, h/2 is the half-height, and $W_{h/2}$ is the width at half-height, for peak 1. W_1 and W_2 are the respective widths of peaks 1 and 2 at the baseline.

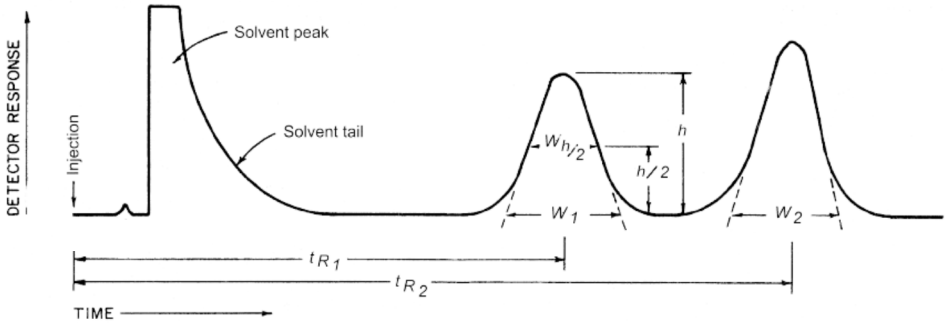


Figure 18: Example of chromatographic separation of two samples (39).

In order to make a quantitative assessment of the compound, a sample with a known amount of the compound of interest is injected and its peak height or peak area is measured (38).

RT is defined as the time elapsed between the injection of the sample and the appearance of the maximum peak response of the eluted sample zone. This parameter may be used for identification since it is a characteristic of each compound. The coincidence of retention times of a sample and a reference substance can be used as a partial criterion in construction of an identity profile (39).

System suitability tests are used to verify that the chromatographic system is adequate for the intended analysis. The tests are based on the concept that the equipment, electronics, analytical operations, and samples analyzed constitute an integral system that can be evaluated as such. Factors that may affect chromatographic behavior include mobile phase, flow rate, column and pressure (39).

Replicate injections of a standard preparation or other standard solutions are compared to determine precision of the method. Unless otherwise specified in the individual monograph, data from five replicate injections of the analyte are used to calculate the relative standard deviation (RSD) (39).

Variability of Cascade Impaction Measurements

Despite cascade impaction can provide information not always available from other particle-sizing methods, it can be time consuming and, because it is complex and expensive to automate remains a largely manual technique, increasing the possibility of analytical error (40) (41). Since many factors not related to product quality may influence the CI measurement outcome, this method has long been recognized in the scientific community as not being very robust (41).

To assure robustness and the desired performance of NGI method, one of the strategies is the development of the method following the Analytical Quality by Design (AQbD) approach.

Analytical Quality by Design

The quality by design (QbD) drug development concept has been used by the pharmaceutical and biopharmaceutical industry over the past decades following the guidance from International Conference on Harmonization (ICH) (42). According to ICH Q8 guidelines QbD is "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management" (35). It is widely used by the pharmaceutical industry to understand the manufacturing process control and drug product quality (42).

Since analytical methods are used in process development and product quality control, their level of importance is so high that if they are poor the results can be inaccurate, resulting in misleading information that may influence the drug development program (42).

ICH guidelines and the USP highlight the need to manage risk during the complete method lifecycle. Applying the QbD approach to analytical methods ensures a controlled risk-based development of a method where quality will be guaranteed (43)(44).

Analytical target profile

In AQbD, the starting point is to define the objectives of the method and analytical target profile (ATP). Each analytical method should have its objective, for example, to be used to support research, process and formulation development, or release and

stability testing for clinical or marketed drugs, quantitative, qualitative, or limit test. ATP describes the method requirements which are expected to be measured (42).

Since ATP is not linked to any particular method, more than one technique can satisfy a single ATP (45). Through various tests, the most suitable analytical technique is chosen and the method can meet the requirements of the intended purpose (42).

Method Design

Method design is a step that allows availability of material (e.g. reagents), sets various experimental conditions and checks feasibility of instruments. Method development strategy includes design of experiments (DoE). It is helpful in risk assessment by acquiring knowledge about existing method and allows for effective control strategies for critical parameters (46).

Risk Assessment

Risk based approach is based on the ICH guideline Q8 and Q9 (46). When each analytical step is examined, risk assessment can be performed based on the step's criticality and impact, providing the analysts a good understanding of the significance of each step and effort required for potential laboratory investigations (42).

There are some tools, such as Fishbone diagrams, failure mode and effect analysis (FMEA), and the prioritization matrix that can help to identify and minimize or avoid potential risks to the method (47).

FMEA

FMEA is a step-by-step approach for identifying all possible failures in a design, manufacturing or assembly process, or a product or service.

Actions are prioritized according to how serious the failure consequences are, how frequently they occur and how easily they can be detected. The purpose of the FMEA is to take actions to eliminate or reduce failures, starting with the highest-priority ones.

To perform this type of analysis it is necessary to determine how serious each effect is - severity rating (S). Severity is usually rated on a scale from 1 to 10, where 1 is insignificant and 10 is catastrophic.

For each failure mode, the potential root causes must be identified and for each cause, the probability rating (P) must be determined. This rating estimates the probability

of failure occurring for that reason during the lifetime of scope. Probability is usually rated on a scale from 1 to 10, where 1 is extremely unlikely and 10 is inevitable.

For each cause, it must be established which are the existing process controls. These controls might prevent the cause from happening, reduce the likelihood that it will happen or detect failure after the cause has already happened but before the customer is affected.

For each control, it must be determined the detection rating (D). This rating estimates how well can the cause or its failure mode be detected after they have happened but before the customer is affected. Detection is usually rated on a scale from 1 to 10, where 1 means the control is absolutely certain to detect the problem and 10 means the control is certain not to detect the problem (or no control exists).

The risk priority number (RPN) is then calculated (S×P×D) and the final results provide guidance for ranking potential failures in the order they should be addressed (48).

Once the risk is assessed it is grouped into three categories:

1. Factors that should be stringently controlled, can be fixed at the time of method development that includes data analysis methods and sample preparation methods;
2. Potential noise factors: variables which are hard to control or are uncontrolled and contribute to inherent variability/error;
3. Factors that can be explored experimentally to determine acceptable ranges (46).

Control Strategy

It is important that method performs as intended and consistently gives accurate results. The method quality control strategy is established from understanding of the criticality of each method parameter in each method attribute. The controls can then be set as procedures or acceptance criteria in the analytical method. (42) One example of this step is system suitability that can be checked and verified time to time by having control over it (46).

Advantages of AQbD

Implementation of QbD principles allows finding optimal separation conditions for development of robust analytical methods with fewer method transfer or failures issues (35).

AQbD is thought as a tool for regulatory flexibility and robust analytics as it explores scientific understanding in method implementation sequences. It ensures a controlled risk-based development of the methods where quality assurance will be guaranteed because working within the Design Space of a specific method can be seen as an adjustment and not a (post approval) change (44)(49).

Benefits of adopting QbD for analytical method:

- This approach gives greater transfer success when method is transferred from research level to quality control department;
- It provides a space for invention of new techniques by continuous improvement throughout life cycle;
- It helps for enhanced understanding of the method;
- Design space concept avoids the post-approval changes which may cause high costs;
- It provides greater compliance with regulatory authorities (46).

Objective

The main objective of this work was to develop a NGI method to characterize an inhalation product using the AQbD approach.

In order to achieve the objective, a group of analysts and laboratory managers that have experience on NGI methods was reunited. Prior to beginning the development, the first aim was to perform a risk assessment in order to evaluate the critical steps of a general NGI method, followed by a FMEA exercise.

Once the FMEA exercise was completed, a workflow was created to summarize the steps of an NGI method development. Firstly, the parameters of an HPLC method to quantify the API were assessed. After that, the formulation components as well as the solvent to recover the API were evaluated. The following step was to define the optimal number of capsules in order to obtain the proper mass balance and to allow the quantification of all the NGI components at an amount superior to the method limit of quantification (LOQ). After the definition of the number of capsules a set of tests was performed to evaluate the influence of equipment components shaking time and shaking type during recovery step, on the results obtained.

While performing the development, the API recovery technique, coating agent, inter-stage losses, mass balance and stability of the solutions were also evaluated.

Chapter II – Materials and Methods

Materials

Solvents: Milli-Q purified water (H_2O), acetonitrile (CH_3CN), methanol (MeOH), ethanol, glacial acetic acid (CH_3COOH), phosphoric acid (H_3PO_4), glycerol 85%;

Reagents: ammonium acetate, sodium dodecyl sulfate (SDS), monobasic ammonium phosphate;

Standard: API X working standard;

Samples: Hypromellose (HPMC) empty capsules, API X capsules (20mg total mass: 1% API, 89% coarse grade lactose and 10% fine grade lactose);

Software: Copley Inhaler Testing Data Analysis Software (CITDAS) was used for data calculation;

Equipment: Critical Flow Controller Model TPK 2000 (Copley Scientific), Vacuum pumps Model HCP5 (Copley Scientific), NGI equipped with IP and PS (Copley Scientific), Flowmeter Model DFM2000 (Copley Scientific), Gentle Rocker MSP4515 (Copley Scientific), DUSA shaker (Copley Scientific), collection tubes, Sonic Branson 8510, Leak test Model 171 (Copley Scientific), Analytical Balance Mettler Toledo, 913 pH meter (Metrohm), DUSA, DUSA filter (Whatman Glass Microfiber filter 47 mm diameter), parafilm, MPA, silicone rubber stoppers, DPI's devices (Plastiape 0111648 – 100 L/min, 0110938 – 60 L/min), HPLC Waters 2487 Dual Wavelength Absorvance Detector.

Methods

Risk Assessment

A team built by analysts and project managers from the development department with experience on the general NGI method identified six process steps, that were well defined in the literature (50), relevant to be considered for further studies:

- Method;
- Measurement;
- Machine;
- Material;
- Man;
- Environment.

After identification of the general parameters, the team elaborated a list of variables (for each step) that could go wrong and affect the outcomes of the method (modes of failures).

Once the variables were identified, by use of FMEA, each one was studied in detail to answer three questions:

- "*What are the potential effects on the customer/patient?";*
- *"What are the potential causes for the failure?";*
- "*What are the existing controls that prevent either the failure itself or its causes?*".

The answer to these questions joined with the team experience, allowed to rank the failures based on their severity, probability of occurrence and detection frequency. At the end of FMEA, it was possible to classify the risk of each failure and to select the method parameters with higher risks.

In order to understand if FMEA results were capable of improve analytical method development, it was selected an old method for API quantification that was no longer used on the routine and it was developed an NGI method. During development, the higher risk variables identified by FMEA were studied to obtain the ideal conditions for the method.

Method I for API quantification

Mobile Phase and dissolution mixture

- **Mobile Phase:** Buffer solution pH 2.7:CH_3CN (48:52 v/v)
- **Dissolution Mixture** – 0.05% H_3PO_4 in CH_3CN:H_2O (50:50 v/v)

Standard solution preparation

Standard stock solution A/B (SSSA/SSSB) - weight 40.0 mg of API X standard to a 100 mL volumetric flask. Dilute to volume with dissolution mixture.

- Working Standard A 1 (WSA 1) - 40.0 μg/mL;
- Working Standard A 2 (WSA 2) - 8.0 μg/mL;
- Working Standard A 3 (WSA 3) - 4.0 μg/mL;
- Working Standard A 4 (WSA 4) - 3.0 μg/mL;
- Working Standard A 5 (WSA 5/LOQ) - 1.2 μg/mL;
- Working Standard B (WSB) - 8.0 μg/mL.

System Suitability

In order to assess HPLC parameters, the operating conditions of Table 2 were set on the equipment.

Table 2: Operating conditions of HPLC.

Detector	Temperature	Column	Others
278 nm	Column Temperature: 25°C	Symmetry C18 250 mm x 4.6 mm 5 µm	Flow: 2.0 mL/min Injection Volume: 100 µL Run Time: 12 min Program: Isocratic

After setting the operating conditions, the system suitability was evaluated by a calibration curve and standard repeatability. The main objective of this step was to understand if the HPLC method was suitable to main peak quantification. At the same time, linearity and precision of the method were evaluated.

Definition of optimum number of capsules

At this stage, a calculation was performed to define the number of capsules to be actuated into the NGI equipment to initiate method development.

In order to perform the referred calculation, some factors were considered:

- Number of formulations/dosage strengths that will be tested – 1;
- LOQ concentration of the HPLC – the same as WSA 5 (C_{WSA5}=1.20 µg/mL);
- Lowest recovery volume initially tested – 5 mL;
- Number of components on NGI setup (according to API recovery) – 13 (combination of 7 stages, MOC, PS, IP, MPA, device and capsule).

The formulation that was tested was composed by 1% API and had a 20 mg fill weight, which means that each capsule had 200 micrograms (µg) of API (LC equals to 200 µg/capsule).

If only one capsule would be actuated and if the dose was fractioned equally among all the NGI components, then the 200 µg of API would be divided in 13 components, e.g. approximately 15.4 µg of API per component. Given that these would be diluted in 5 mL (lowest volume initially tested on API recovery), the final concentration of the API recovered from the component would be approximately 3 µg/mL.

Considering that LOQ concentration is 1.20 µg/mL, the API concentration would be higher so the number of capsules to be actuated should be one. Despite the calculations performed, it is generally known that dose is not equally fractioned among all the NGI components, so probably some components would have a concentration of API recovered lower than LOQ. Because of this, on the initial analysis it was tested the actuation of only one capsule and then the actuation of two capsules.

Method II for API quantification

An alternative method to Method I, taking into account a USP monograph, was also used to quantify the API.

Mobile phase preparation

Mobile Phase: MeOH:0.01M Monobasic ammonium phosphate buffer pH 3.5:CH_3CN (50:35:15 v/v)

Dissolution mixture: equal to method I because API is soluble in it.

Standard solution preparation

The same stock standard solutions presented at method I were used because dissolution mixture is the same. However, the dilutions performed (presented on the Results and Discussion chapter) were a result of the areas obtained with the new operating conditions.

System Suitability

In order to assess HPLC parameters, the operating conditions of Table 3 were set on the equipment.

Table 3: Operating conditions of HPLC.

Detector	Temperature	Column	Others
239 nm	Column Temperature: 40°C	Symmetry C18 250 mm x 4.6 mm 5µm	Flow: 1.5 mL/min Injection Volume: 20 µL Run Time: 15 min Program: Isocratic

Recovery Techniques

One of the most important parameters that have to be assessed during the development is the API recovery technique from the NGI.

The solvent needed to recover all product and therefore API from the NGI stages and components should be defined and should also take into account the recovery procedure (e.g. type and time of agitation), the component capacity, the quantitation method and LOQ.

The same dissolution mixture was used for both reference standards and recovery of API of NGI components.

To evaluate the volume of recovery solvent needed, a table (Table 4) was elaborated with a range of volumes that may be added to each component.

Table 4: Range of volumes possible to be tested at recovery procedure.

NGI (stages and components)	Volume (mL)
Capsules	10-50 mL
Device	10-30 mL
MPA	10-20 mL
IP	15-50 mL
PS	35-200 mL
Stage 1	5-25 mL
Stage 2	5-20 mL
Stage 3	5-20 mL
Stage 4	5-20 mL
Stage 5	5-20 mL
Stage 6	5-20 mL
Stage 7	5-20 mL
MOC	5-10 mL

In the present work the type and time of agitation will be studied according with Table 5 procedure.

Table 5: Time and type of agitation at the recovery procedure.

Component	Shaking manually		Inverting		Gentle Rocker		Sonication	
	1 minute	5 minutes	1 minute	5 minutes	5 minutes	10 minutes	5 minutes	10 minutes
IP	Yes	Yes	Yes	Yes	No	No	Yes	Yes
PS	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Stages	No	No	No	No	Yes	Yes	Yes	Yes

Coating agent

In order to prevent collection of the particles on the wrong downstream stage a coating agent was selected.

Since there were a big amount of coating agents that could be used, it was decided to use the most commonly used in the company, a solution of 1% glycerol in ethanol.

The influence of the coating agent was studied by changing the evaporation time (5 and 30 minutes) and also by performing an NGI without coating.

Setup of NGI

Prior to initiating NGI test, all the equipment were dried and well cleaned. Then, a set of cups was coated (all the surface must be covered) with a thin and uniform film.

After ethanol evaporation a leak test was performed before each test. In the leak test, the initial pressure was 4kPa and the final pressure was recorded after 20 seconds. The NGI test was only performed when the pressure variation was less than 100 Pa/s.

In order to perform the flow rate setup, PS and IP were assembled on the impactor. Flow rate setup was conducted with Q_{test}=100 L/min or Q_{test}=60 L/min (depending on the device used). The desired flow rate (±5%) was achieved after connecting a calibrated flow meter to the IP and adjusting the critical flow controller. After recording Q_{test}, P2 and P3, it was evaluated if P3/P2 is ≤ 0.5. If this criterion passed, the timer in the flow controller was adjusted (t ± 5%) so that the two-way solenoid valve opened for the necessary duration to draw 4 L of air through the inhaler for the established flow rate, where:

$$t(s) = \frac{4(L) \times 60(s)}{Q_{test}(L/min)}$$

Cascade impaction testing and sample recovery

The NGI test began with the addition of 15 mL of dissolution mixture to the PS. Then, the flow controller was adjusted to run the test, selecting x shots (correspondent to x capsules), with t seconds actuation. After inserting the capsule on the device, it was punctured to allow powder distribution. The device was attached to the inlet of the mouthpiece adapter and connected to the IP. Only after those steps the vacuum pump was switched on to draw air for t seconds. The following procedure, used for samples preparation, was based on the recovery of the API at the 13 components (figure 19).

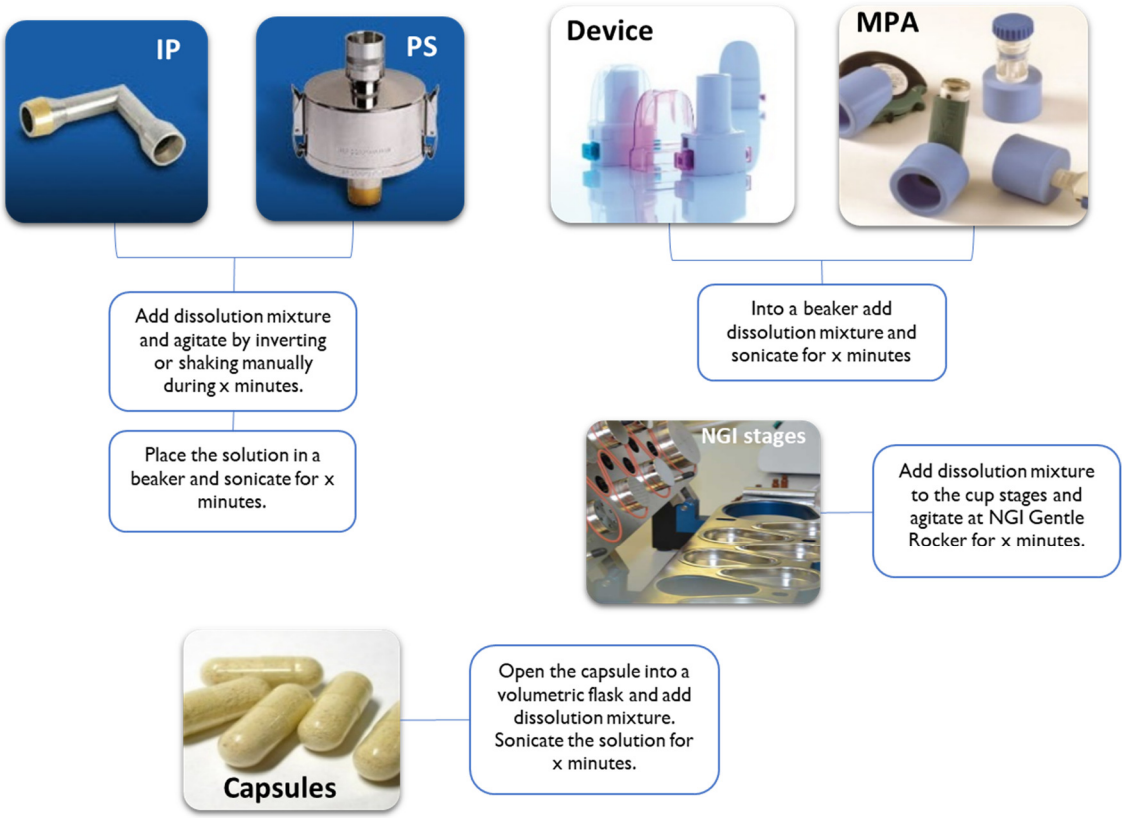


Figure 19: Recovery procedure for API extraction.

Stability of standards and sample solutions

Additionally, it was decided to study the solutions' stability. The solutions (standards and samples in recovery matrix on coated stages) were injected 24 (T24 h) and 48 hours (T48 h) after the first injection on HPLC (T0 h). Thermal stability was also studied since the solutions were stored at T2-8°C and at the autosampler (at room temperature).

Since volume of sample solutions was not enough, stability at room temperature after 48h was not studied.

To evaluate the results obtained, the following equation was used to evaluate the % of difference between T0, T24 h and T48 h:

$$\%\, difference = \frac{|Area_{inicial} - Area_{T24h/48h}|}{Area_{inicial}} \times 100$$

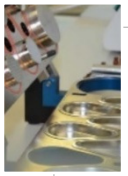
Chapter III - Results and Discussion

Risk Assessment

By analysis of each step of the pharmacopoeial NGI method, the team has chosen the factors that were considered to have significance on the results or that impact was not studied until this work (figure 20).

Method

- Parameter determination variability
- Linearity
- API recovery from capsule
- MOC less than Limit of Quantification
- HPLC related problems
- Interstage losses
- PS and IP - agitation type and time
- Volume of solvent in PS before analysis
- Coating drying time
- Capsule actuation time
- Coating aplication mode
- Type and thickness of coating
- Solubilities
- Agitation time



Machine

- Cleaning and drying
- Power shutdown
- Leak between PS+IP+NGI
- Length and thickness of tubing
- Microdefect on equipment
- Malfunction of equipment while testing
- Grouping of PS+IP+cups
- Calibration/maintenance

Man

- Experience of user
- Analyst fatigue/stress
- Vials loss
- Handling of device
- Switch on stage recuperation
- Alignment of device
- Vial identification
- Training

Environment

- Temperature
- Relative humidity
- Electrostatic

Material

- Filtration effect
- Moisture sensitivity/ storage conditions
- Batch variability
- Solution stability
- Compatibility between parafilm/stoppers and API
- Wrong MPA
- PS use (dependent on excipient presence)
- Incorrect dissolution mixture
- Wrong device
- Device resistance
- Empty capsule batch + type of capsule + frequency of empty capsule change
- Type of material used (glass vs. plastic)

Measurement

- Bad application of parafilm and stopper
- Time between weighing and actuation of capsules
- Flow change between DUSA and NGI
- Cups attached to O-rings
- Do not puncture the capsule
- Mistake at leak test
- Contaminations
- Number of capsule actuations
- Bad application of DUSA filter
- Weighing of capsule
- Recuperation volumes
- Time between actuation and recuperation

Figure 20: Risk assessment analysis.

FMEA

After performing the risk assessment analysis (figure 20), each potential failure was classified based on severity, probability and detection rate. Table 6 presents the critical attributes with RPN higher than 100, which require study or a corrective action.

Table 6: Critical attributes identified by FMEA that require study or a corrective action.

Process	**Failures**	**Classification (before corrections)**			
Process Steps	**Mode** *"What could go wrong?"*	**S** Severity (1-10) **If ≥ 9 Act!**	**P** Probability (1-10) **If ≥ 7 and S ≥7 Act!**	**D** Detection (1-10)	Risk Priority Number (RPN) RPN = P x S x D **If ≥ 100 Act!**
Method	Interstage losses	**7**	4	6	**168**
	Type and time of agitation for PS and IP	6	4	8	**192**
	Coating drying time	6	**7**	5	**210**
	Type and thickness of coating	**7**	**7**	6	**294**
	Agitation time	**7**	**7**	7	**343**
Measurement	Contaminations	**8**	**8**	3	**192**
	Time between capsule actuation and API recovery	**7**	**7**	7	**343**
Environment	Temperature	**7**	**8**	7	**392**
	Relative humidity	**7**	**8**	7	**392**
	Electrostatic	6	**7**	7	**294**
Machine	Leak between PS, IP and NGI	6	3	6	**108**
	Length/ thickness of tubing	3	**8**	5	**120**
Material	Moisture sensitivity/ storage conditions	**7**	**8**	5	**280**

By analysis of table 6, the majority of the factors identified should be stringently controlled and can be fixed at the time of method development. However, there are some noise factors, like contamination and the time between capsule actuation and API recovery that are hard to control and contribute to inherent variability of the measurement.

The potential failures related to environment can be explored experimentally to determine acceptable ranges, but in the present work they were not studied, as these should be studied along with the formulation development work.

The critical attributes related to the machine can be controlled by performing a leak test before each analysis and by homogenizing the tubing for all NGI stations. At last there are the factors related to the moisture used, which can be controlled by studying the formulation and its ideal storage conditions.

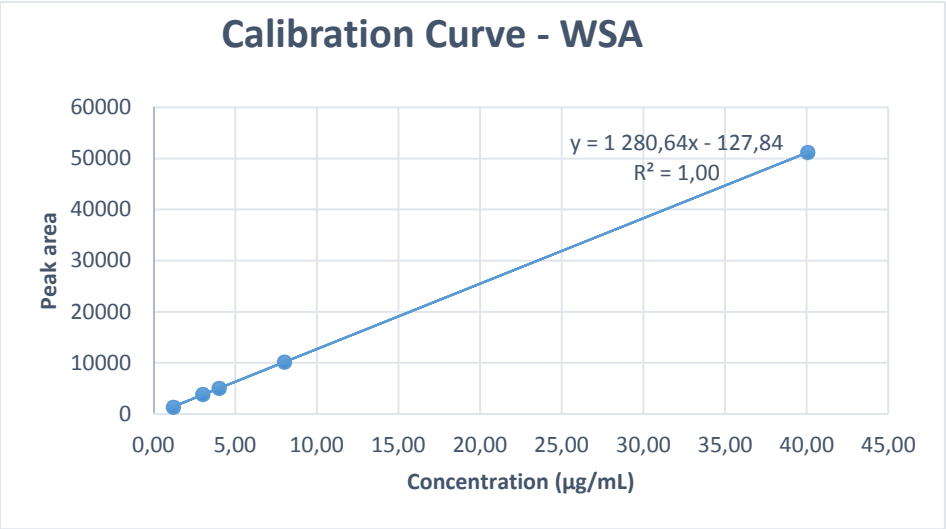
At the end of the FMEA, it was decided that only the critical attributes related to the method were going to be studied. To understand the impact of each attribute, the method development was divided in several sequential and individual steps leading to a final method.

All the present work was based on the study of the critical attributes, such as agitation time (of all the NGI components), type and thickness of coating and its drying time. Additionally, at the end of this work it is expected to optimize steps in order to have full knowledge of the method and to make its development easier.

Method I

System suitability

In order to begin the experiment, a system suitability was performed to evaluate the precision and linearity of the HPLC method already existent. After preparation of SSSA, SSSB and respective dilutions, a linear regression was performed. As it can be seen on graphic 1, concentration of solutions was set as the independent variable and peak areas as the dependent ones.



Graphic 1: Calibration curve of WSA with equation chart and coefficient of determination (r^2).

By analysis of graphic 1, it can be verified that the method has a good linearity. System precision was evaluated by analyzing %RSD of WSB five injections (present on table 7).

Table 7: WSB five injections - peak areas, RT and %RSD (Method I).

	Peak Area	**RT (min)**
1st injection	10245	5.758
2nd injection	10071	5.757
3th injection	10173	5.756
4th injection	10361	5.759
5th injection	10217	5.760
Average	10213	5.758
%RSD	1.0	0.03

At table 7 can be verified that the method has a good precision, since the % of RSD obtained through the five injections of WSB was 1.0% on peak area and 0.03% on retention time of main peak.

The final criteria to evaluate if system was able to realize a proper quantification of API is the D-Check between standards (WSA 2 and WSB average). This parameter is obtained by the following equation:

$$D - Check = \frac{Average\ area\ WSB}{WSA\ 2\ peak\ area} \times \frac{Weight\ SSSA}{Weight\ SSSB} \times 100$$

Applying the results of weightings, WSA 2 peak area and average area of WSB (at table 7) on the previous equation, a D-Check of 100% was obtained. Thought all the work, sample solutions only were injected if the following acceptance criteria were met:

- D-check: 98%-102%;
- % RSD on five injections of WSB ≤2%.

Definition of optimum number of capsules

The beginning of the experiment was performed with one capsule. During all the work, the capsules were weighed prior to analysis and at the end to obtain the real capsule fill weight.

In order to evaluate if shaking time on Gentle Rocker and sonication time influenced the obtained results, after the recovery of the previous samples (dilution volumes presented on table 8), the remaining solutions continued on agitation for an additional 5 minutes. At the end, stage cups were on agitation for 10 minutes and MPA, IP, PS, device and capsules were on sonication for 10 minutes. Results are shown in table 9 and graphic 2.

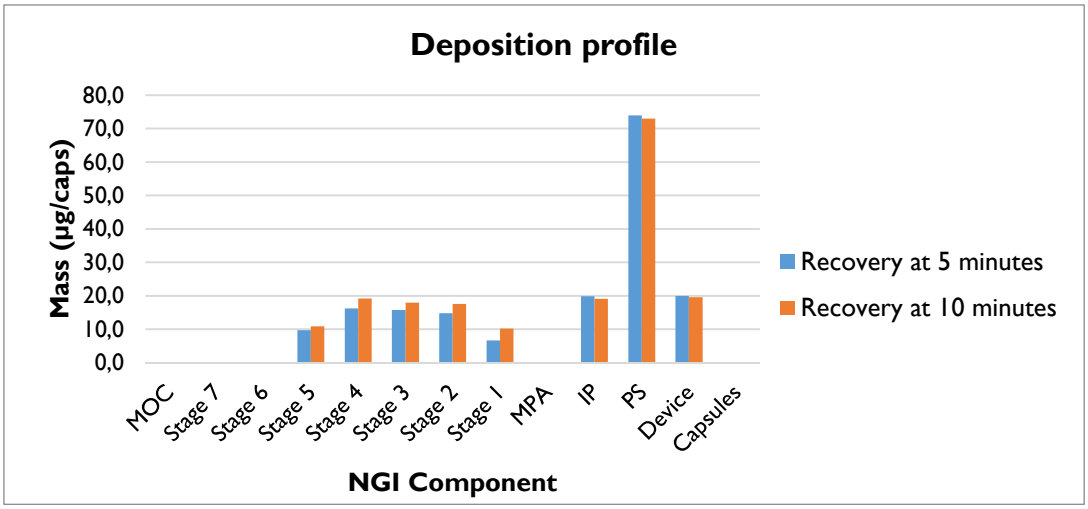
Table 8: Dilution volumes used to recover API at sample solutions.

Dilution volume (mL)						
MOC	**Stage 1 – Stage 7**	**PS**	**IP**	**MPA**	**Device**	**Capsules**
5	5	35 (15 mL+20 mL)	15	10	10	10

Table 9: Results of NGI with one capsule - Gentle Rocker/ Sonication 5 and 10 minutes (Method I).

	Concentration (µg/mL)		Mass (µg/capsule)	
Agitation time	**5'**	**10'**	**5'**	**10'**
MOC	< LOQ	< LOQ	0.000	0.000
Stage 7	< LOQ	< LOQ	0.000	0.000
Stage 6	< LOQ	< LOQ	0.000	0.000
Stage 5	1.950	2.187	9.752	10.935
Stage 4	3.250	3.846	16.249	19.228
Stage 3	3.156	3.600	15.781	17.998
Stage 2	2.962	3.526	14.812	17.631
Stage 1	1.336	2.039	6.680	10.193
PS	2.113	2.086	73.951	72.994
IP	1.326	1.274	19.887	19.114
MPA	< LOQ	< LOQ	0.000	0.000
Device	1.997	1.962	19.965	19.622
Capsules	< LOQ	< LOQ	0.000	0.000
Mass balance (%LC)			88.538	93.858
Aerodynamic properties	**ED (µg)**		157.112	168.093
	FPD (µg)		52.251	60.349
	FPF (%)		33.257	35.902
	MMAD (µm)		2.597	2.692
	GSD		2.011	2.128

Graphic 2: Deposition profile comparison between API recovered after 5 and 10 minutes.



By analysis of table 9 and graphic 2, it can be seen that the mass obtained on the stages increased from 5 minutes of agitation on Gentle Rocker to 10 minutes. The

aerodynamic properties presented in table 9 showed a difference in terms of FPD and FPF which means that the particles with a cut-off lower than 5µm had a higher mass when the stages were on agitation 10 minutes instead of 5.

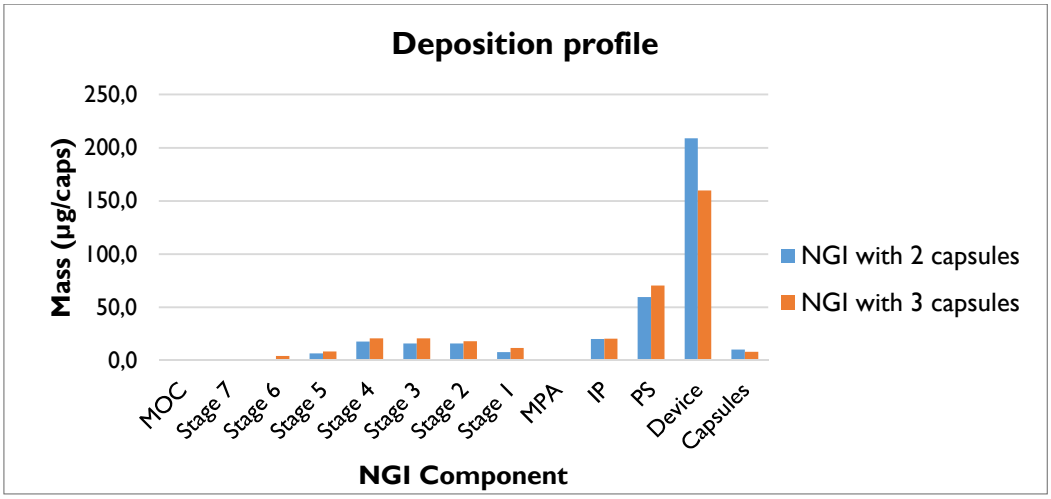
These results showed that 5 minutes of agitation on Gentle Rocker were not enough to fully dissolution of the particles on the recovery solvent. So, from this point forward all the samples were recovered after 10 minutes of agitation/sonication.

Despite the mass balance was in accordance with the recommendation of FDA, some stages (stage 6, 7 and MOC) did not have a concentration higher than LOQ suggesting that the number of capsules actuated was not enough.

Since the dilution volume used was already low (and could not be lower), two experiments with 2 and 3 capsules were performed (results are shown on table 10 and graphic 3).

Table 10: Results of the NGIs performed with 2 and 3 capsules (Method I).

	Concentration [(1)] (µg/mL)		**Mass (µg/capsule)**	
Number of capsules	**2 capsules**	**3 capsules**	**2 capsules**	**3 capsules**
MOC	< LOQ	< LOQ	0.000	0.000
Stage 7	< LOQ	< LOQ	0.000	0.000
Stage 6	< LOQ	2.591	0.000	4.319
Stage 5	2.667	5.192	6.667	8.653
Stage 4	7.086	12.467	17.714	20.779
Stage 3	6.387	12.565	15.967	20.942
Stage 2	6.362	10.875	15.902	18.126
Stage 1	3.171	7.113	7.926	11.856
PS	3.418	6.045	59.820	70.521
IP	2.718	4.123	20.383	20.615
MPA	< LOQ	< LOQ	0.000	0.000
Device	41.776	47.975	208.878	159.916
Capsules	2.047	2.427	10.237	8.091
Mass balance (%LC)			181.749	171.909
Aerodynamic properties	**ED (µg)**		144.382	175.811
	FPD (µg)		51.741	67.547
	FPF (%)		35.836	38.420
	MMAD (µm)		2.722	2.626
	GSD		2.020	2.184
y=1194.82x+247.75 (r^2=1)				



Graphic 3: Comparison between the deposition profile of NGI performed with 2 and 3 capsules.

Evaluating the previous results of table 10 and graphic 3, it can be seen that the peak of device had a concentration much higher than what was expected (when compared to table 9 and graphic 2). The cause of this unexpected result can be the interference of the plastic material of the device.

Despite the abnormal mass balance result, the aerodynamic properties obtained are not dependent of the device mass result. So, by comparison of the FPD and FPF, it can be verified that the NGI performed with 3 capsules had, as expected, higher values than the NGI performed with 2 capsules. Despite the higher mass on the main stages of the NGI, stages like MOC, stage 7 and stage 6 (with lower cut-off diameters) still not presented a peak with concentration higher than LOQ.

To investigate the root cause of the abnormal area obtained at the device peak (see table 10) a new device without the capsule was placed into a beaker and 10 mL of dissolution mixture were added. The beaker was agitated and an aliquot was transferred to a HPLC vial. This procedure was repeated with the only difference being the sonication of the device during 10 minutes.

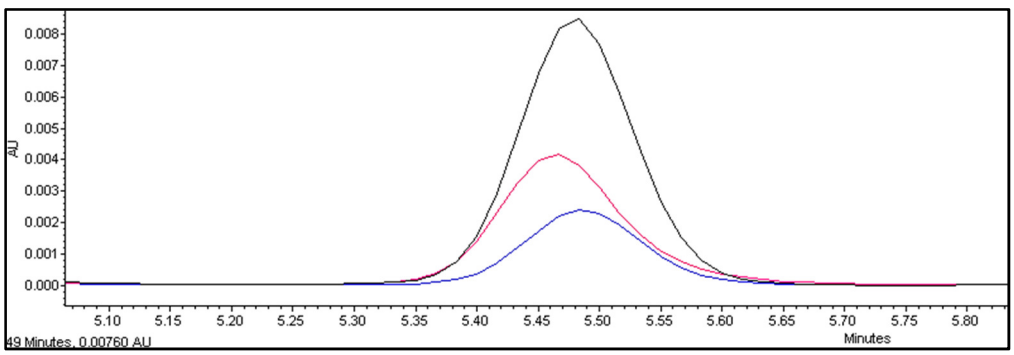


Figure 21: Overlay of the chromatograms obtained by injecting device with API (dark line), device without API sonicated 10 minutes (pink line) and device without API and without sonication (blue line).

After overlaying the chromatograms correspondent to the peak of device with API with the ones correspondent to the device without API (figure 21) it can be concluded that they have the same retention time, giving a wrong idea about the area of API remaining on the device at the end of the NGI. As can be seen on figure 21 the interfering peak increases when the device is sonicated, so the solution adopted was to not sonicate the device from this point forward.

Since at the first NGI performed (with one capsule) the peak of the device had a different retention time, the cause of the interferent peak was probably related to the HPLC method. This occurred because when the HPLC method was developed, the device used was different so the selectivity of the method was not a problem before.

Despite the problem at this method, it was performed two NGIs with 5 and 10 capsules (maximum of capsules allowed) and the dilution volumes used were the same as referred on table 8 with exception of capsules that were diluted on a 20 mL volumetric flask. The results are present on table 11.

Table 11: Results of the NGIs performed with 5 and 10 capsules (Method I).

	Concentration (µg/mL)		**Mass (µg/capsule)**	
Number of capsules	**5** [(1)]	**10** [(2)]	**5**	**10**
MOC	< LOQ	< LOQ	0.000	0.000
Stage 7	< LOQ	< LOQ	0.000	0.000
Stage 6	< LOQ	2.789	0.000	1.394
Stage 5	7.833	12.714	7.833	6.357
Stage 4	17.865	35.581	17.865	17.790
Stage 3	19.131	36.785	19.131	18.393
Stage 2	18.630	38.252	18.630	19.126
Stage 1	11.299	22.065	11.299	11.032
PS	10.521	20.757	73.646	72.651
IP	7.449	14.011	22.347	21.017
MPA	< LOQ	4.019	0.000	4.019
Device	6.012	24.046	12.024	24.046
Capsules	2.609	3.732	10.438	7.463
Mass balance (%LC)			96.607	101.644
Aerodynamic properties	**ED (µg)**		170.751	171.779
	FPD (µg)		58.024	57.511
	FPF (%)		33.981	33.480
	MMAD (µm)		2.881	2.901
	GSD		2.077	2.050

(1) $y=1267.66x+274.05$ $R^2=1$ (2) $y=1276.61x+31.24$ $R^2=1$

As it can be seen on table 11, even with the maximum number of capsules a concentration higher than LOQ was not obtained in some components. Since method I was not suitable for routine analysis due to its lack of selectivity and specificity, method II was selected to evaluate if the problems presented at the device injections continued to occur.

Method II

The same vials of standards and samples (correspondent to the NGI performed with 10 capsules which results were presented on table 11) were re-injected on the HPLC with the operating conditions of method II and its correspondent mobile phase. Results are present at table 12.

Table 12: Results of the re-injection of standards and samples correspondent to NGI performed with 10 capsules. (Method II)

	Concentration (1) (µg/mL)	**Mass (µg/capsule)**
MOC	< LOQ	0.000
Stage 7	< LOQ	0.000
Stage 6	3.9405	1.970
Stage 5	18.0747	9.037
Stage 4	14.1534	7.077
Stage 3	5.0346	2.517
Stage 2	< LOQ	0.000
Stage 1	2.3289	1.164
PS	30.1896	105.664
IP	20.4980	30.747
MPA	5.5468	5.547
Device	11.9800	11.980
Capsules	5.4623	10.925
Mass balance (%LC)		93.314
(1) **$y=23453.15x+3859.38$ ($r^2=1$)**		

At table 12, it can be seen that the correlation coefficient obtained was 1, which means that this method also has a good linearity. By injection of five WSB, a %RSD of

0.7% on peak area and 0.2% on retention time was obtained, indicating a good precision of the method.

Since the retention time of API X changed in the new method used, the problems at the device injection (due to the interference peak) disappeared, as it can be seen on figure 22, which means that Method II has a good selectivity.

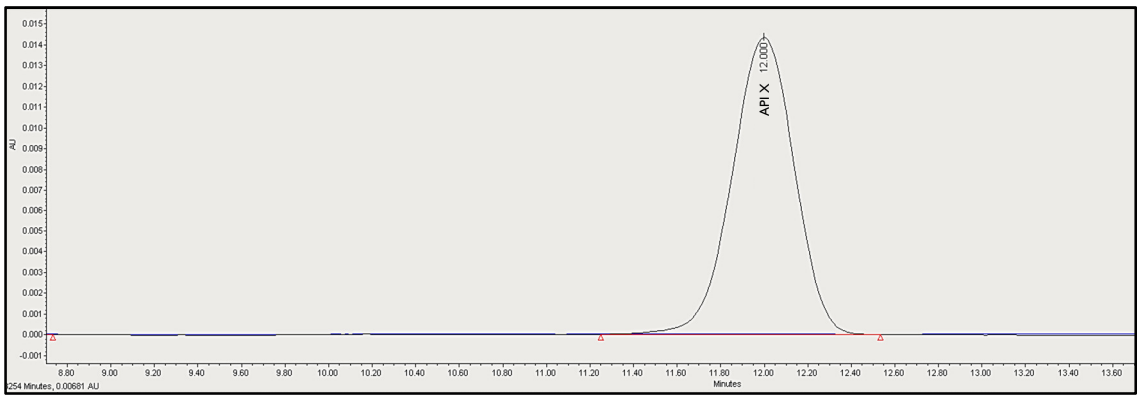


Figure 22: Overlay of chromatogram obtained by injection of device with API (NGI with 10 capsules) and without API (Method II).

Also, with the new method it can be observed big differences on the chromatograms obtained. The differences consist essentially on a better baseline (with less noise) and a better resolution of the API X peak on solutions with a smaller concentration (see figure 23).

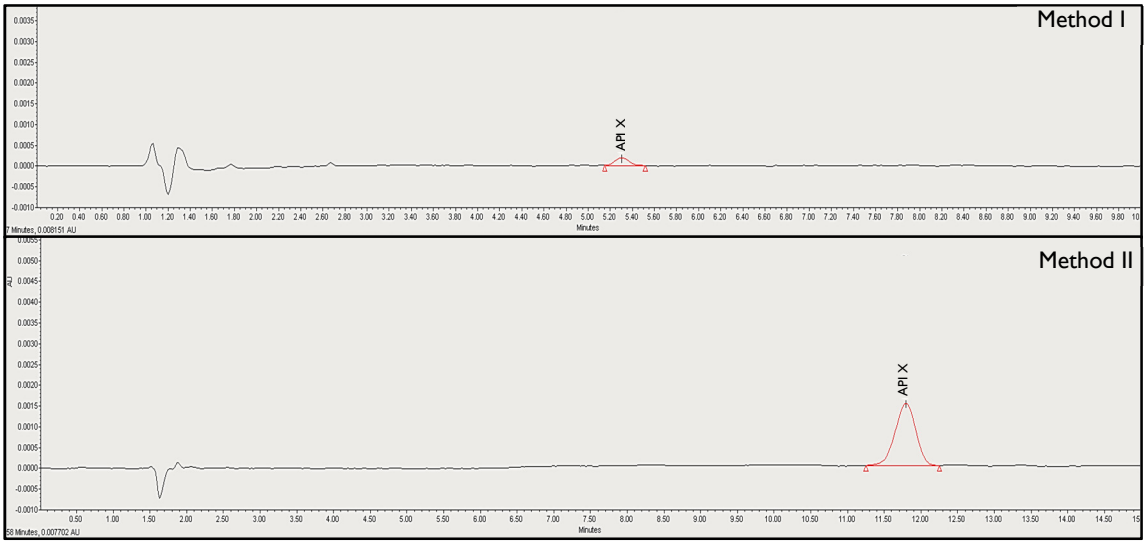


Figure 23: Chromatograms obtained by injection of WSA 5 with method I and method II.

System Suitability

At evaluation of the results obtained with method II a change on LOQ concentration was considered in order to decrease the area obtained and the number of capsules used at the NGI. The following new dilutions of the stock standard solutions were performed:

- Working Standard A 1 (WSA 1) - 20.0 µg/mL;
- Working Standard A 2 (WSA 2) – 4.0 µg/mL;
- Working Standard A 3 (WSA 3) - 2.0 µg/mL;
- Working Standard A 4 (WSA 4) - 0.6 µg/mL;
- Working Standard A 5 (WSA 5) - 0.16 µg/mL;
- Working Standard A 6 (WSA 6/LOQ) - 0.08 µg/mL;
- Working Standard B (WSB) - 4.0 µg/mL.

Definition of optimum number of capsules

At table 12 it can be seen that with 10 capsules discharged, the API X concentration on the various components of the NGI is too high for the new calibration curve. Two experiments with 2 and 5 capsules were performed in order to evaluate the difference between them. The recovery procedure was made after 10 minutes of agitation and sonication and the dilution volumes used were the same as referred on table 8. The results are shown on table 13.

Table 13: Results of the NGI performed with 2 and 5 capsules (Method II).

	Concentration [1] (µg/mL)		**Mass (µg/capsule)**	
Number of capsules	**2 caps**	**5 caps**	**2 caps**	**5 caps**
MOC	< LOQ	< LOQ	0.000	0.000
Stage 7	0.107	0.890	0.267	0.890
Stage 6	0.838	1.946	2.094	1.946
Stage 5	2.214	6.046	5.536	6.046
Stage 4	6.641	15.977	16.602	15.977
Stage 3	6.728	19.327	16.821	19.327
Stage 2	7.292	18.233	18.229	18.233
Stage 1	3.786	11.151	9.465	11.151
PS	4.451	11.714	77.889	81.996
IP	2.893	7.017	21.698	21.052
MPA	0.751	1.336	3.755	2.671

Device	2.405	5.657	12.025	11.315
Capsules	1.303	3.834	6.515	7.667
Total mass (µg/capsule)			189.881	197.986
Mass balance (%LC)			95.448	99.136
Aerodynamic properties	**ED (µg)**		171.534	179.068
	FPD (µg)		54.121	56.937
	FPF (%)		31.551	31.797
	MMAD (µm)		2.865	2.901
	GSD		2.006	2.072
y=23486.20x+2638.11 (r^2=1)				

By evaluating table 13 it can be seen that for both experiments, MOC has a concentration bellow LOQ while all other components have a similar mass per capsule. With the results obtained, was observed that the number of capsules did not have a major influence in the concentration of API X on MOC. Also, by comparison of aerodynamic properties, all the parameters have very similar results which led to the decision that from this point forward, the number of capsules to be tested with method II is 2.

Influence of shaking time and type

Until this step of the work the recovery procedure of IP and PS was made by shaking them manually during 1 minute. To evaluate if shaking time and type have influence on the result obtained three additional experiments were performed (shaking manually 5 minutes, inverting 1 minute and inverting 5 minutes). The results can be seen on table 14.

Table 14: Results of the NGIs performed with different type and time of shaking (Method II).

	Concentration [1] (µg/mL)			**Mass (µg/capsule)**		
Type and time of shaking	***Shake 5min***	***Invert 1min***	***Invert 5min***	***Shake 5min***	***Invert 1min***	***Invert 5min***
MOC	<LOQ	<LOQ	<LOQ	0.000	0.000	0.000
Stage 7	0.372	0.308	0.225	0.931	0.769	0.563
Stage 6	1.111	0.764	0.971	2.779	1.911	2.426
Stage 5	2.134	2.382	2.183	5.336	5.956	5.458
Stage 4	6.202	5.968	5.700	15.506	14.920	14.250
Stage 3	6.571	6.420	6.133	16.427	16.049	15.333

Stage 2	7.335	7.178	7.741	18.338	17.944	19.352
Stage 1	4.425	3.924	3.554	11.063	9.811	8.884
PS	3.852	3.965	4.371	67.414	69.388	76.493
IP	3.064	3.017	3.158	22.977	22.631	23.686
MPA	0.342	0.452	0.432	1.712	2.261	2.158
Device	3.190	3.534	2.835	15.952	17.668	14.174
Capsules	1.592	1.527	1.235	7.958	7.637	6.175
Total mass (µg/capsule)				185.374	185.928	187.936
Mass balance (%LC)				92.687	92.964	93.968
Aerodynamic properties	**ED (µg)**			161.649	160.806	167.775
	FPD (µg)			53.680	52.149	51.701
	FPF (%)			33.208	32.430	30.815
	MMAD (µm)			2.936	2.911	2.980
	GSD			2.079	2.024	1.921
(1) **y=23486.20x+2638.11 (r^2=1)**						

In order to evaluate the influence of shaking time and type the following equation was applied to the previous results and the table 15 was obtained.

$$\%\text{Mass of PS/IP} = \frac{Mass\ of\ IP\ or\ PS}{Total\ mass} \times 100 \quad \text{(mass expressed in µg/capsule)}$$

Table 15: Results of the % of mass present at PS and IP depending on the type and time of agitation.

	PS	**IP**
Shaking manually 1min (1)	40.9%	11.4%
Shaking manually 5min	36.2%	12.3%
Inverting 1min	37.1%	12.1%
Inverting 5min	40.5%	12.5%
(1) Mass of IP and PS present at table 15.		

By comparison of the values present in table 15, it can be concluded that shaking time and type do not have a major influence on the obtained results indicating that 1 minute of shaking manually is enough for the recovery procedure.

Evaluation of % RSD

To move forward with the development, the following step was to perform three runs with the same method conditions. Despite the use of devices at 100 L/min until this phase of development, there was a rupture of company stock. To overcome that

problem, devices of the same brand (Plastiape) at 60 L/min were tested and the results are present on table 16.

Table 16: Results of the three runs performed at the same conditions.

	Concentration [(1)] (µg/mL)			Mass (µg/capsule)		
	Run #1	**Run #2**	**Run #3**	**Run #1**	**Run #2**	**Run #3**
MOC	<LOQ	<LOQ	<LOQ	0.000	0.000	0.000
Stage 7	0.136	0.196	0.363	0.339	0.490	0.908
Stage 6	0.884	0.918	0.703	2.209	2.294	1.756
Stage 5	3.533	3.127	3.931	8.832	7.817	9.827
Stage 4	8.275	8.481	7.760	20.687	21.202	19.401
Stage 3	7.348	6.862	6.592	18.371	17.154	16.481
Stage 2	4.837	4.647	4.954	12.094	11.618	12.386
Stage 1	2.159	2.051	2.186	5.398	5.127	5.465
PS	3.975	3.962	3.867	69.558	69.335	67.668
IP	2.356	2.471	2.261	17.668	18.535	16.957
MPA	0.493	0.587	0.623	2.465	2.936	3.113
Device	3.772	3.212	3.178	18.860	16.059	15.888
Capsules	1.255	1.368	1.188	6.276	6.841	5.941
Mass balance (%LC)				91.379	89.703	87.895
Aerodynamic properties	**ED (µg)**			157.230	156.113	153.568
	FPD (µg)			53.363	51.789	51.360
	FPF (%)			33.939	33.174	33.445
	MMAD (µm)			2.952	2.896	2.915
	GSD			1.985	2.008	2.056
(1) **$y=30329.37x+1914.27$ ($r^2=1$)**						

As it can be seen in table 16, despite the use of a different device, mass balance continues to be in accordance with acceptance criteria defined on the USP and the aerodynamic properties are quite similar to those verified with 100 L/min devices.

Table 17: %RSD of APSD parameters obtained by CITDAS software.

%RSD				
ED (µg)	**FPD (µg)**	**FPF (%)**	**MMAD (µm)**	**GSD**
0.98	1.65	0.94	0.80	1.47

In table 17 are presented the results of RSD % between the three runs. All the results are inferior to 20% (the initial acceptance criteria defined) which means that the method has a good repeatability.

Evaluation of coating influence

The following step was the evaluation of coating influence on particle size distribution. Until this point the coating used was always the same (1% glycerol in ethanol) and since its thickness was a difficult parameter to control, the evaluation was performed by changing the time necessary for ethanol evaporation. Three NGIs were performed: without coating, 5 minutes after coating application and 30 minutes after coating application.

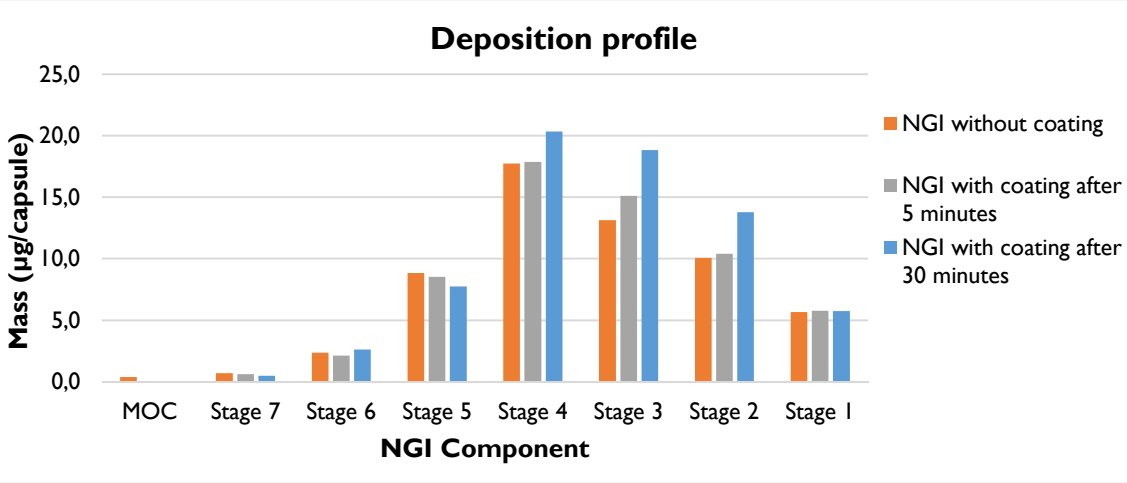
Results are presented in table 18 and the deposition profile of the NGI stages are present in graphic 4.

Table 18: Results of the tests performed without coating and with different coating evaporation times (5 and 30 minutes).

	Concentration (µg/mL)			Mass (µg/capsule)		
	Without Coating [1]	Coating 5 min [1]	Coating 30 min [2]	Without Coating	Coating 5 min	Coating 30 min
MOC	0.149	<LOQ	<LOQ	0.373	0.000	0.000
Stage 7	0.275	0.252	0.197	0.688	0.630	0.492
Stage 6	0.950	0.847	1.050	2.374	2.118	2.625
Stage 5	3.531	3.410	3.095	8.827	8.526	7.738
Stage 4	7.090	7.143	8.139	17.725	17.858	20.348
Stage 3	5.256	6.045	7.534	13.141	15.113	18.834
Stage 2	4.026	4.155	5.512	10.065	10.389	13.781
Stage 1	2.269	2.305	2.301	5.672	5.762	5.752
PS	3.952	4.067	4.344	69.167	71.178	76.025
IP	2.945	3.192	2.507	22.090	23.942	18.803
MPA	0.972	0.429	0.581	4.859	2.145	2.903
Device	1.569	3.864	2.927	7.846	19.321	14.634
Capsules	1.139	1.271	1.414	5.696	6.355	7.069
Mass balance (%LC)				84.261	91.669	94.502
Aerodynamic properties	**ED (µg)**			154.981	157.838	167.103
	FPD (µg)			45.533	46.837	53.593
	FPF (%)			29.380	29.674	32.072

	MMAD (µm)	2.783	2.915	3.058
	GSD	2.231	2.129	1.979
(1) **y=26298.34x-56.33 (r^2=1)** (2) **y=30605.88x-484.71 (r^2=1)**				

Graphic 4: Comparison of the NGIs performed without coating and with different times of coating solution evaporation.



When comparing results present on table 18, like mass balance, ED, FPD and FPF it can be concluded that the evaporation of the coating agent is a factor with a major influence on the method performance. After 30 minutes of evaporation, the coating solution was able to attach more particles with a cut-off below 5µm than on the other tests.

As it can be seen in graphic 4, the increase on evaporation time of the coating agent has an influence on the deposition profile in the various stages of the NGI. When comparing (table 18 and graphic 4) the three NGI's performed it can be concluded that when the coating is dry the API has a major tendency to attach to the intermediary stages. The results of the NGI performed without coating prove the theory that dry particles are prone to bounce off the collection surface and consequently get re-entrained in the air stream, being collected on the subsequent stage and being incorrectly sized (like it was observed on MOC).

For the first time, it was obtained a GSD < 2 which means that with a proper coating agent, the formulation has tendency to monodisperse simulating the deposition of the powder on the desired site of action. MMAD obtained was similar through the work and had acceptable values since it was inferior to 5 µm meaning that this

formulation with the device in study has a predominant deposition on the smaller airways.

In order to evaluate the presence of wall losses, an additional test was added at the end of the NGI. That test consists of recovering the remaining API present in the interstage. To perform the recovery of interstage nozzles, a new set of cups without coating was introduced and the interstage used on the NGI test was detached. Then, 15 mL of dissolution mixture were added over stage 1 of interstage to remove any API present in the nozzles and the solution collected in the cup was used to wash the remaining stages of the interstage. The final solution present in the MOC was injected in the HPLC and compared with the other NGIs with different coating evaporation times.

On table 19, we can evaluate the interstage wall losses on each of the NGI performed.

Table 19: Results of interstage wall losses without coating solution and with different evaporation times when the solution was present.

Interstage Wall Losses		
Without coating	**Coating 5min**	**Coating 30min**
3.597 µg/capsule (2.13%)	1.760 µg/capsule (0.96%)	3.194 µg/capsule (1.69%)

By analyzing table 19, it can be concluded that all the NGIs had interstage wall losses inferior to 5% (when compared with the total mass per capsule), that is in line with USP requirements and ensure a proper mass balance. Since this parameter had an acceptable result, the coating agent used is suitable for the API in use.

Stability of standards and sample solutions

To obtain additional information about the standards and samples solutions, a thermal-stability was performed and the results are presented in tables 20 and 21, respectively.

Table 20: Stability results of standard solutions.

	T0 h	T24 h (T2-8°C)		T48 h (T2-8°C)		T24 h (room temperature)	
	Area	Area	% Difference	Area	% Difference	Area	% Difference
WSA 6	2820	2725	3.4%	2493	11.6%	2514	10.9%
WSA 5	5040	5145	2.1%	4942	1.9%	5001	0.8%
WSA 4	18310	18386	0.4%	18178	0.7%	18334	0.1%
WSA 3	58909	59071	0.3%	57583	2.3%	58448	0.8%
WSA 2	121923	123159	1.0%	120361	1.3%	121986	0.1%
WSA 1	612681	615876	0.5%	601155	1.9%	606273	1.0%

By analysis of table 20, it can be concluded that according with the company acceptance criteria (% difference between T0 and Tx less or equal than 2% with exception of LOQ which criteria is 30% instead of 2%), standard solutions are stable for 24 hours at room temperature conditions and 48 hours when stored at T2-8°C.

Table 21: Stability results of sample solutions.

	T0 h	T24 h (T2-8°C)		T48 h (T2-8°C)		T24 h (room temperature)	
	Area	Area	% Difference	Area	% Difference	Area	% Difference
MOC	838	857	2,3%	892	6,4%	800	4,5%
Stage 7	5533	5512	0,4%	5371	2,9%	5422	2,0%
Stage 6	31648	32268	2,0%	31703	0,2%	31670	0,1%
Stage 5	94246	93312	1,0%	93410	0,9%	93963	0,3%
Stage 4	248624	248151	0,2%	246427	0,9%	247875	0,3%
Stage 3	230088	227536	1,1%	226192	1,7%	227942	0,9%
Stage 2	168225	166601	1,0%	165245	1,8%	166236	1,2%
Stage 1	69933	70457	0,7%	70602	1,0%	70200	0,4%

By analyzing table 21, it can be concluded that sample solutions have a validity of 24 hours when stored at room temperature or at T2-8°C.

Final considerations

During method development, using AQbD approach, it is important to understand which are the critical process steps that need to be controlled and monitored.

Using AQbD approach on this work, it was possible to optimize the initial development steps, preventing the study of one factor at a time in the future, saving time and material resources.

At figure 24, it can be seen the fundamental steps that are essential on a NGI method development. As an additional step, it was established that content uniformity of capsules should also be studied during NGI method development, helping in understanding the product/formulation variability.

Table 22 is a result of the AQbD approach and summarizes the optimized instructions to perform each step of the process.

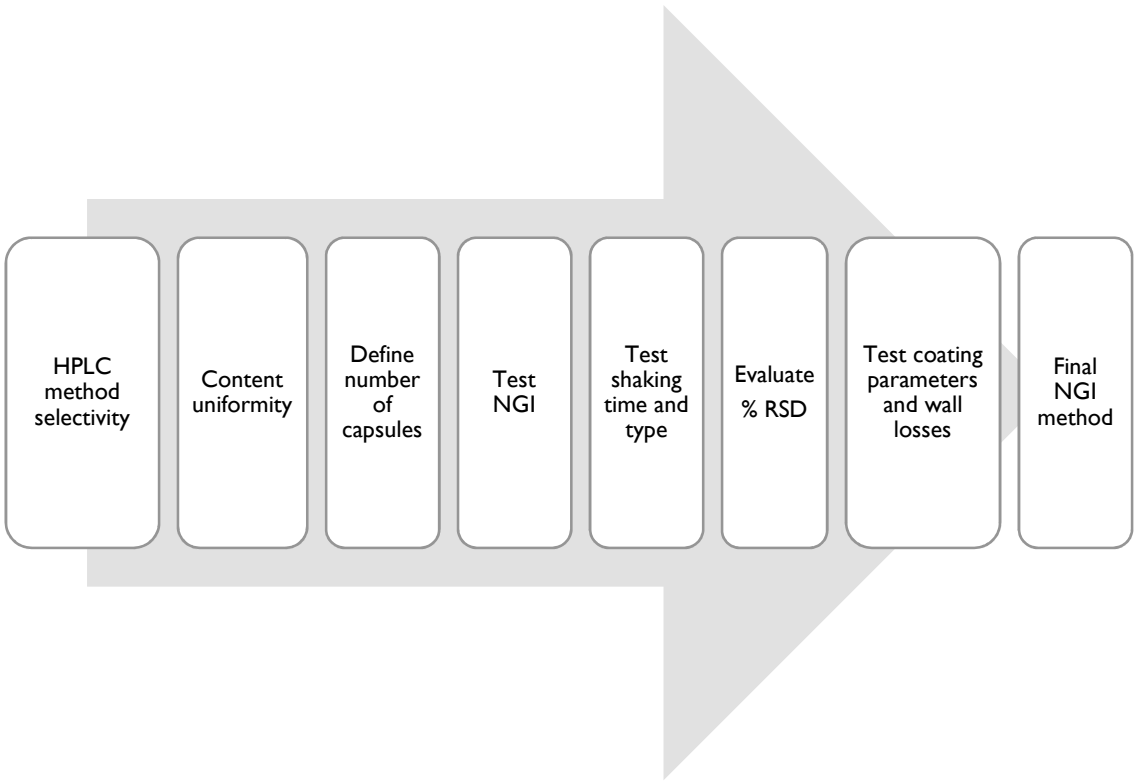


Figure 24: Summary of the fundamental steps necessary to develop a NGI method.

Table 22: Instructions for each process step of NGI method development.

Process step	Instructions
Selectivity evaluation	During method development the selectivity of the HPLC method (to quantify API) should be demonstrated for the following: • Coating agent solution, when applicable; • Filters (DUSA filters, syringe filters, etc); • All excipients; • Capsules and other devices for drug administration; • Inhalation equipment material (plastic, rubber stoppers); • MPA.
Definition of number of capsules	At this stage, perform theoretical calculation to define the number of capsules to be actuated into the NGI equipment to initiate method development. The theoretical calculation should consider: • Number of formulations/dosage strengths that will be tested; • LOQ concentration of the HPLC; • Lowest volume initially tested; • Number of components on NGI setup from which to have API recovered.
Test NGI	Begin the experiment with the minimum volume allowed at each NGI stage/component. Test shaking time and type can be evaluated when studying the optimum number of capsules to speed the process. Basically, the recovery of the API from cup stages and NGI components can be performed after 5 and10 minutes on NGI gentle rocker and sonicator, respectively. For IP and PS, the process can be performed by taking an aliquot after 5 minutes of shaking manually/inverting and after 10 minutes. At the end, results can be compared to evaluate the optimum time and type of agitation. By evaluation of API concentration at NGI stages/components, it may be observed that API concentration at MOC stage is not influenced by the number of capsules tested, which leads to a concentration bellow LOQ. However, if mass balance and aerodynamic properties are within the acceptable criteria, the next step can be performed.
% RSD determination	Three NGI tests must be performed and % RSD must be evaluated for the following parameters: ED, FPD, FPF, MMAD and GSD. In case of acceptance criteria failure, recovery volumes must be adjusted. While performing the three NGI tests, testing of wall losses can be performed.
Coating and wall losses testing	Test coating parameters and wall losses evaluation can be performed at the same test to speed the process and to evaluate coating impact on interstage wall losses.

Chapter IV - Conclusion

The present study was based on the development of an NGI method to characterize one inhalation product using the AQbD approach.

At the beginning of the work, was performed a risk assessment to evaluate the critical steps of the method, followed by a FMEA. The results obtained allowed to select a group of variables with higher risk associated to the method, like: agitation time, presence of interstage losses, type and thickness of coating as well as its drying time.

When HPLC parameters were assessed it was found that, despite being linear and precise, the first selected method for quantification of the API was not selective due to the presence of interfering peaks on the device. A backup method was then used and the results showed a good linearity, precision, selectivity and repeatability.

During the definition of the optimum number of capsules, it was verified that all the stages and other components had a concentration able to be quantified (with concentration higher than LOQ) with exception of MOC. Since MOC is the stage with a lower cut-off diameter, is easy to understand that the particles have more difficulty to reach it.

By performing a series of tests to evaluate the influence of shaking time and type it can be concluded that the agitation of cup stages on Gentle Rocker and the time of sonication had a major influence on the results. However, the tests performed with different agitation times and types of PS and IP had results quite similar, meaning that the impact of agitation on these components was insignificant.

To evaluate the influence of coating agent, three tests were performed and it can be concluded that in its absence the particles tend do deposit on the wrong stage due to rebounce effect. It was also concluded that a longer evaporation time of the coating agent had advantages on the aerodynamic properties obtained, like higher ED and FPD values. Also, at study of inter-stage losses, the NGI performed without coating was the one that presented higher losses. This can be easily explained since without coating, particles have higher difficulty to attach to the cup stages, being accumulated on the interstage nozzles. However, it can be concluded that the coating agent chosen was appropriate to the API in study since the acceptance criteria defined by the USP (inter-stage losses inferior to 5%) was fulfilled.

Mass balance is also an important factor for the regulatory authorities and by performing an analysis of all the results, it can be concluded that the results fulfill the

acceptance criteria with exception of the test performed without coating agent (%LC<85%).

At the end, stability of the solutions and uniformity of delivered dose were assured. Despite it was proven that the capsules used through all the work had a uniform content, delivered dose was lower than what was expected indicating that the adhesion properties of the powder influence the results obtained.

The method development steps were simplified and optimized. An example of this optimization was on the evaluation of agitation time by performing a single test but recovering the samples at different times of agitation.

It can be concluded that, development of a method with AQbD has many advantages since it increases the robustness and the understanding of the method. However, it may be of interest to study the impact of environmental conditions on the results obtained.

Although the objective was achieved, NGI methods continue to have many procedural steps that are dependent on the analyst, which may contribute to variability. A way to reduce this is the semi-automation of the process by acquiring, for example, a NGI coater (standardizes amount, uniformity and method of application of coating agent) and a NGI assistant (places a known quantity of solvent in each cup, gently agitates the contents in order to dissolve the active drug in solvent and then places a representative sample of solution from each of the cups into HPLC vials).

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