Statistical tools to control the internal lubricant content of inhalation grade HPMC capsules

Guillermo Ayala[a], Fernando Díez[b], María T. Gassó[c], Brian E. Jones[d]

[a]*Department of Statistics and O.R. University of Valencia Avda. Vicent Andrés Estellés, 1, 46100-Burjasot (Spain)*
[b]*Qualicaps Europe, S.A.U. Avda. de Valdelatas 4. 28108-Alcobendas, Madrid (Spain)*
[c]*Institute of Multidisciplinar Mathematics and Department of Mathematics Polythecnical University of Valencia, Camino de Veras/n 46022. Valencia (Spain)*
[d]*School of Pharmacy & Pharmaceutical Sciences, Cardiff University, Cardiff, UK*

Abstract

Pulmonary delivery is being becoming a popular route for delivering active pharmaceutical ingredients that cannot be administered through by the standard oral route, as well as offering an improved alternative to the parenteral route. The use of hard capsules in dry powder inhalers (DPIs) to deliver formulations to the lung has been in use since 1970, and recently there has been an interest in changing from metered-dose devices to capsule-based ones because they are simple to formulate, cheap to manufacture, and patient-friendly. The original inhalation grade hard capsules were made from gelatin, which becomes brittle when exposed to low humidities. Inhalation grade hypromellose (HPMC, carrageenan gelling agent) has been developed in the last few years to overcome come this problem and has been shown to have better aerosolization properties.

Hard capsules are made by a dipping process in which a surface lubricant is an essential processing aid for removing dried capsule shells from the manufacturing pins. This lubricant has been shown to have an effect on powder retention in capsules that are used for the inhalation of medicines.

The capsule manufacturing process depends on many factors. The response is a function giving the the internal lubricant content (ILC) from 0 to 48 hours. Two of them were chosen by experts in order to evaluate their influ-

Email addresses: Guillermo.Ayala@uv.es (Guillermo Ayala), Fdiez@qualicaps.es (Fernando Díez), mgasso@mat.upv.es (María T. Gassó), JonesBE2@cardiff.ac.uk (Brian E. Jones)

ence on ILC: pin location and the pump flow. A global and a local functional analysis of variance have been used in order to compare the mean functions observed under the two experimental factors just considered.

Additionally we want to know if the mean ILC is between the tolerance limits. A bootstrap confidence region is used to estimate local confidence regions of the response.

Keywords: HPMC capsules, internal lubricant, aerosolization, dry powder inhaler (DPI), drug retention, functional data analysis.

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1. Introduction

Hard capsules are manufactured in a continuous process on large automatic machines (figure 1). They are formed on stainless steel mould pins mounted in-line onto metal strips (bars).There are different sets of bars to make the caps and bodies of each size of capsule. Groups of bars are dipped in to a temperature controlled container, called a dip pan, containing a warm aqueous solution of the polymer, either gelatin or hypromellose (HPMC). Films are formed on the mould pins most commonly by a gelation process that relies on the temperature difference between the cold pin and the hot solution. This is an inherent property of gelatin solutions and HPMC solutions are formulated to gel by the addition of a network former such as carrageenan and potassium chloride as a promoter (2). After dipping, the bars are raised out of the dip-pan and are rotated end over end to improve the film distribution on the pins as they are transferred from the lower level of the machine to the upper one. At this point the film have set and are no longer mobile. Groups of bars are moved by hydraulic pushers through a series of drying kilns, which use large volumes of controlled humidity and temperature to dry the films. At the end of the upper level the bars are transferred to the lower level and are moved back to the front-end of the machine. When the pins emerged from the kilns they are dried to a level, $> 16\%$ for gelatin and $> 6.5\%$ for HPMC (inhalation grade), which is just above the upper level of the standard moisture content specification. The dried films adhere strongly to the pin. Pairs of bars, one with cap and one body, are selected from each side of the machine and enter a central automatic section. The next part of the process is to strip them from the pins using soft metal jaws and the ILC is a critical factor enabling this to occur without capsule damage. If insufficient is used the capsule shells will split

during removal. The mould pins are then cleaned and lubricated. The lubricant is a proprietary mixture pharmaceutical grade excipients and is different for each capsule manufacturer and their compositions are registered in the companies Drug Master File. Lubricant is loaded into a pump, the flow rate from which can be adjusted using a pressure valve. The lubricant is applied to a circular foam pad that transfers a sufficient quantity to the pins as they pass underneath. The pin bars are moved towards the centre of the machine and the pins are inserted into rotating circular tubes lined with felt pads. These clean the pins and spread the lubricant evenly over their surface. The pads are changed at regular intervals to avoid a build-up and saturation with the lubricant [5, 6, 7, 8, 9].

Several papers have described the influence of ILC on aerosolization [12, 13]. The reference [13] showed that there is an optimum ILC range to obtain good powder release from capsules as measured by their emitted dose and fine particle fraction [13]. They suggested that the effect could be related to the roughness of capsule internal surface.

Ayala et al. in a recent work proposed a statistical model that could be used to control the internal lubricant content of capsules within the required limits during the manufacturing process [1]. In this paper a new approach using functional data analysis will be proposed. The internal lubricant content during the whole experimental interval is considered as a whole function i.e. not just values at a given times. If we consider as response this function then the natural statistical framework is functional data analysis. In this paper we use this approach.

2. Materials and methods

HPMC inhalation grade capsules were manufactured by Qualicaps Europe (Spain, Madrid). Capsule pin lubricant was manufactured with pharmaceutical grade materials using the formulation registered in the USA drug master file, N14765 (Qualicaps Europe S.A.U.). Internal lubricant concentration was evaluated by determining methyl oleate (MO), which was taken as a marker for the lubricant content.

Samples of 11 capsules were weighed in a glass vial. Five ml of Hexane: chloroform, 60:40 (v/v) extraction solvent containing 10mg/l of the internal standard were added to the samples. The vial was sonicated during one hour in an ultrasonic bath; then 100l of the extract was transferred into a 2ml vial for derivatizasion using 50l of Trimethyl sulfonium hydroxide(TMSH). The

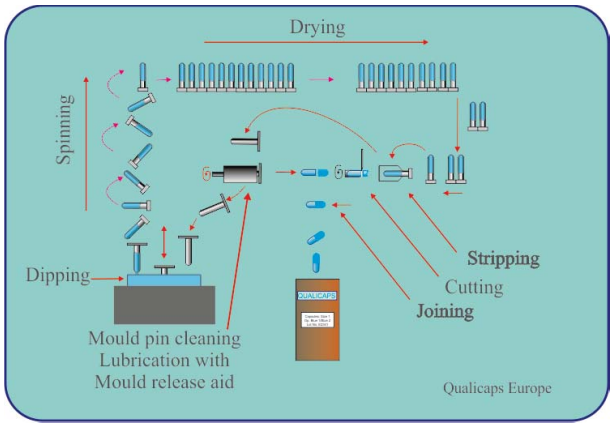

Figure 1: Manufacturing process.

resulting methyl esters were analyzed by Gas chromatography-mass spectrometry (GCMS). The MO was identified by GCMS and quantified using an internal calibration method using six points in the 0.5-20mg/kg concentration range. One microliter of the derivatized MO was injected in the splitless mode in the GCMS [10].

The internal lubricant content was studied as a function of different factors in the production process; previously selected by people experienced in capsule manufacturing. The three factors considered to be important were: ILC application pump-flow rate, pin position on a bar in the dipping pan where capsule are formed and the time interval from the last change of the ILC application shells. This resulted in three levels for pump-flow (low, medium and high), for pin position (Bar 4, Pin 1-2-3 and Pin 28-29-30) and time from the change of application shells. Two replicated samples were taken for each condition that resulted in 432 samples.

3. Experimental setup

An experiment was designed and performed in order to evaluate possible factors affecting to the internal content of lubricant. This is the major aim of this research. Three factors were considered: the pump flow, the pin location and the time delayed. The variables, as usual, can be classified in response, internal lubricant content in this case, and predictors (controled in order to evaluate their effect over this response). The data set used in [1] is reanalyzed here.

Each experimental setup was replicated twice. As the functional datum for trial i is a set of discrete measured values $\{(t_j, y_j)\}_{j=1,\ldots,m}$ where t_j is the j-th time and y_j is the observed internal lubricant content. Our first task was to convert these values to a function $x_i(t)$ computable for any time t. Each trial has the same time interval associated, $[0, T]$. In what follows we will refer to any of these $x_i(t)$ as $x(t)$. We would like to point out that we did not use an interpolation process because we assumed that the discrete values may include some observational error. Instead, we have used a smoothing technique to transform the raw data $\{(t_j, y_j)\}_{j=1,\ldots,m}$ to a function $x(t)$ possessing a certain number of derivatives. Actually, we have obtained them by means of a least squares fitting method. Such a method determines the best choice of the coefficients c_k, $k = 1, \ldots, K$, in the representation of the function as a linear combination of K basis functions. From several possibilities we chose a polynomial spline basis $\{\Phi_k(t)\}_{k=1,\ldots,K}$, where each

$\Phi_k(t)$ is a piecewise cubic function, and we obtained the coefficients c_k of the expansion $x(t) = \sum_{k=1}^{K} c_k \Phi_k(t)$ by minimizing the least squares criterion $SMSSE(y/c) = \sum_{j=1}^{n}(y_j - \sum_{k=1}^{K} c_k \Phi_k(t_j))^2$ [11, chap. 3, pp. 44-51]. Concerning the number of functions K in the spline basis, we decided to set it to $K = 6$.

Figure 2 displays the original data and the fitted functional data by distinguishing the pump-flow. From now on, we will work with the fitted functional data instead of the original values observed.

4. Results

We previously analyzed this data by means of an analysis of covariance. However, this is not a completely right approach. A closer view of the lubricant content shows that the ILC's depends each other for close times. The needed hypothesis of independence for different times is not tenable and this model does not take into account this constraint. The response is not just the observed ILC at a given time. The response is the function along the whole time interval. This is the real response, a function and not a single value.

We have observed a function (lubricant content) along time at a discrete set of times and a functional data has been fitted for each function. This is the point.

We have two experimental factors: pump-flow and pin location. We are going to evaluate each factor separately. Let us denote $x_{ij}(t)$ for $t \in [0, T]$ with $i = 1, \ldots, I$ and $j = 1, \ldots, n_i$. Note that $x_{ij}(t)$ is the functional data fitted for the the i-the group to be compared ($I = 3$) and j-th observation of this group ($n_i = 6$ for each i).

4.1. Global functional anova

We are dealing with random functions. Our major aim is to evaluate if there exists some difference between the different groups defined by pump-flow (respectively pin location). The null hypothesis could be formulated as: all groups of functions share a common mean function against the alternative hypothesis where the mean functions are different.

It has been used a functional generalization of the anova test proposed in [3]. The well known F value defined as the division of the between and

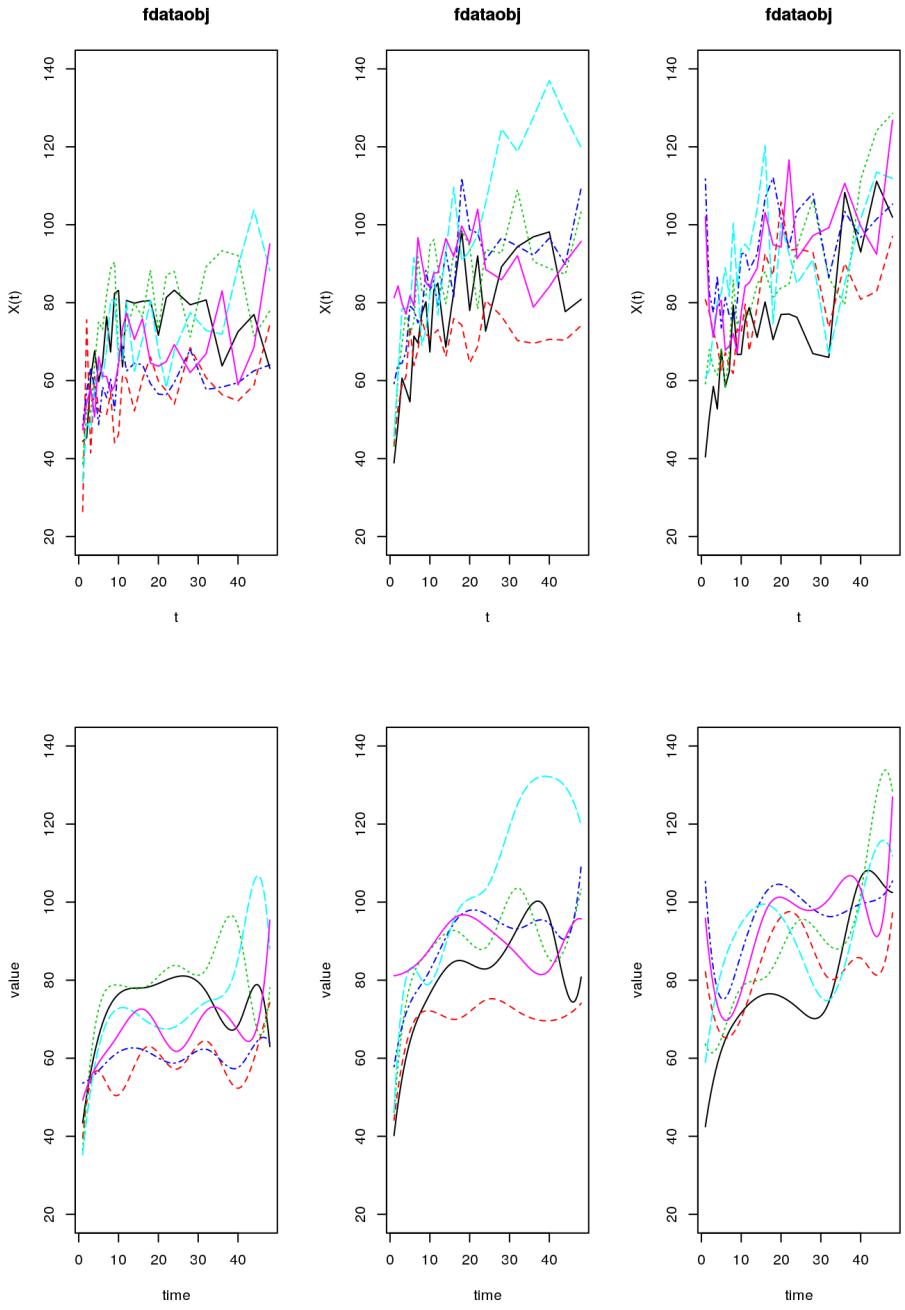

Figure 2: Original data (first row) and the fitted functional data (second row). Each column corresponds with a different pump-flow: low, medium and high from left to right.

within mean squares is replaced by

$$\frac{\sum_{i=1}^{I} n_i \int_0^T (x_{i\cdot}(t) - \bar{x}_{\cdot\cdot}(t))^2 dt}{\sum_{i=1}^{I} \sum_{j=1}^{n_i} \int_0^T (x_{ij}(t) - \bar{x}_{i\cdot}(t))^2 dt} \tag{1}$$

where $\bar{x}_{i\cdot}(t) = \frac{1}{n_i} \sum_{j=1}^{n_i} x_{ij}(t)$ and $\bar{x}_{\cdot\cdot}(t) = \frac{1}{n} \sum_{i=1}^{I} \sum_{j=1}^{n_i} x_{ij}(t)$ where $n = \sum_{i=1}^{I} n_i$.

The classic anova test compare the between and within sum of squares. In this ratio appear these sum of squares just deleting the integral. A large value of this ratio corresponds with a false null hypothesis of a common mean. Now we replace the numbers by functions and the square of their difference by the integral of the difference of the functions. It is a direct generalization. Again, a large value of this ratio will correspond to different mean funcions. We have no distributional assumptions about the random behaviour of the fitted functional data. We can use a randomization test instead of an approximate asymptotic distribution. The functions are randomly asigned to the groups. The same statistic is calculated for each random asignment. The randomization p-value is just the proportion of random statistics greater than the statistic originally observed. It has been used 100 randomizations. The observed p-values appear in table 1. The rows labeled ALL correspond to the comparison between all groups. Pairwise comparisons have been performed using the same test and the corresponding p-values observed appear accordingly labeled. No significant different mean functions are observed for the different groups defined by pin location. We have high p-values greater for instance than 0.05. In particular, the global p-value is 0.31. However, there is a significant p-value for the global comparisons corresponding to pump-flow. A significant difference is observed when compare high vs low pump-flow and low vs medium pump-flow. The only non-significant comparison correspond to high vs medium pump-flow.

4.2. Local functional anova

We have seen that there exists different mean functions for different pump flows. This is a global picture. However, we would like to evaluate similarly around the neighborhood of a given time t_0. We are going to replace the original function x_{ij} by its product with a kernel function defined from -1 to 1, K,

$$x_{ij}(t) K\Big(\frac{t - t_0}{h}\Big), \tag{2}$$

Table 1: Randomization p-values for pump-flow and pin location.

Pump-flow		
	ALL	0.00
	High-Low	0.00
	High-Medium	0.36
	Low-Medium	0.00
Pin location		
	ALL	0.31
	Bar 4 - Pin 1	0.15
	Bar 4 - Pin 28	0.14
	Pin 1 - Pin 28	0.73

where h is the bandwidth. An Epanechnikov kernel function has been used with $h = 3$ hours. The analysis in section 4.1 is repeated to these new functions using a discrete set of times t_0, actually, the same times used in the original experimental setup. Note that we have multiple comparisons for different times and different pairwise comparisons. In order to control the false discoveries we have applied a Benjamini-Hochberg correction Benjamini and Hochberg [2] to the observed p-values. Figure 3 displays the adjusted p-values estimated.

Again, no difference is observed along the time interval when we compare the high and medium pum-flows. A greater difference is observed for the comparisons between low vs medium and high vs low pump-flows. Comparing low and medium pump-flows, we have a significant different (local) mean function from 2 hours up to almost 40. For the comparison between high and low pump-flows a significant different (local) mean function is observed from 0 to one hour, from 12 to 29 hours and from 39 to the end of the time interval, 48 hours.

No local significant difference can be observed for pin location as expected from the previous section.

In the manufacturing process, there is a target value equal to 100 and a tolerance interval from 50 to 150 ppm. It is important to display the estimated mean functions by taking into account these limits. We have estimated these mean functions using a bootstrap method proposed in [4]. First, if we do not consider the different pump flows we can estimate some kind of global mean function. It is shown in figure 4 (top-left). The other three plots correspond to the estimated mean function when the pump flow fac-

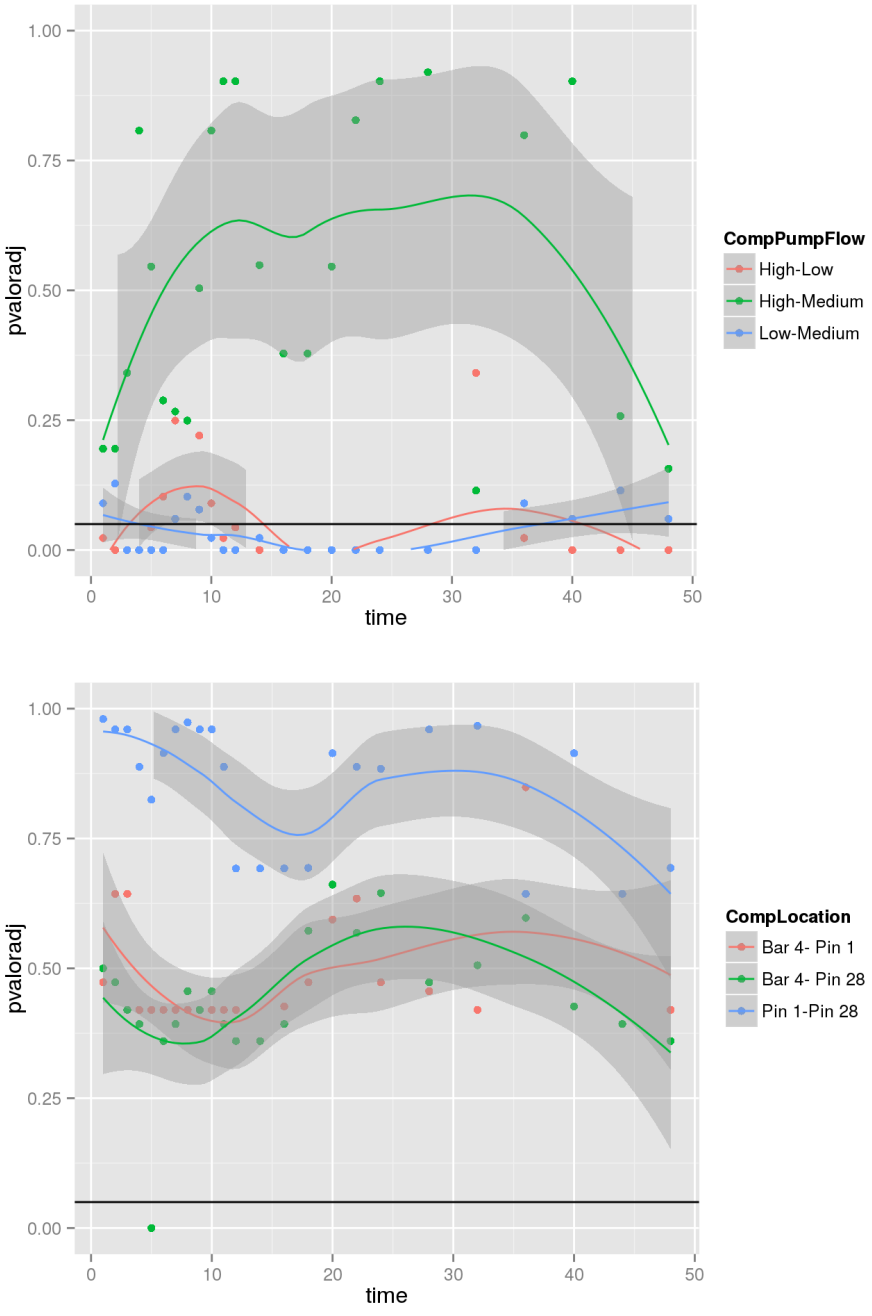

Figure 3: Adjusted local anova p-values for pump-flow (top) and pin location (bottom).

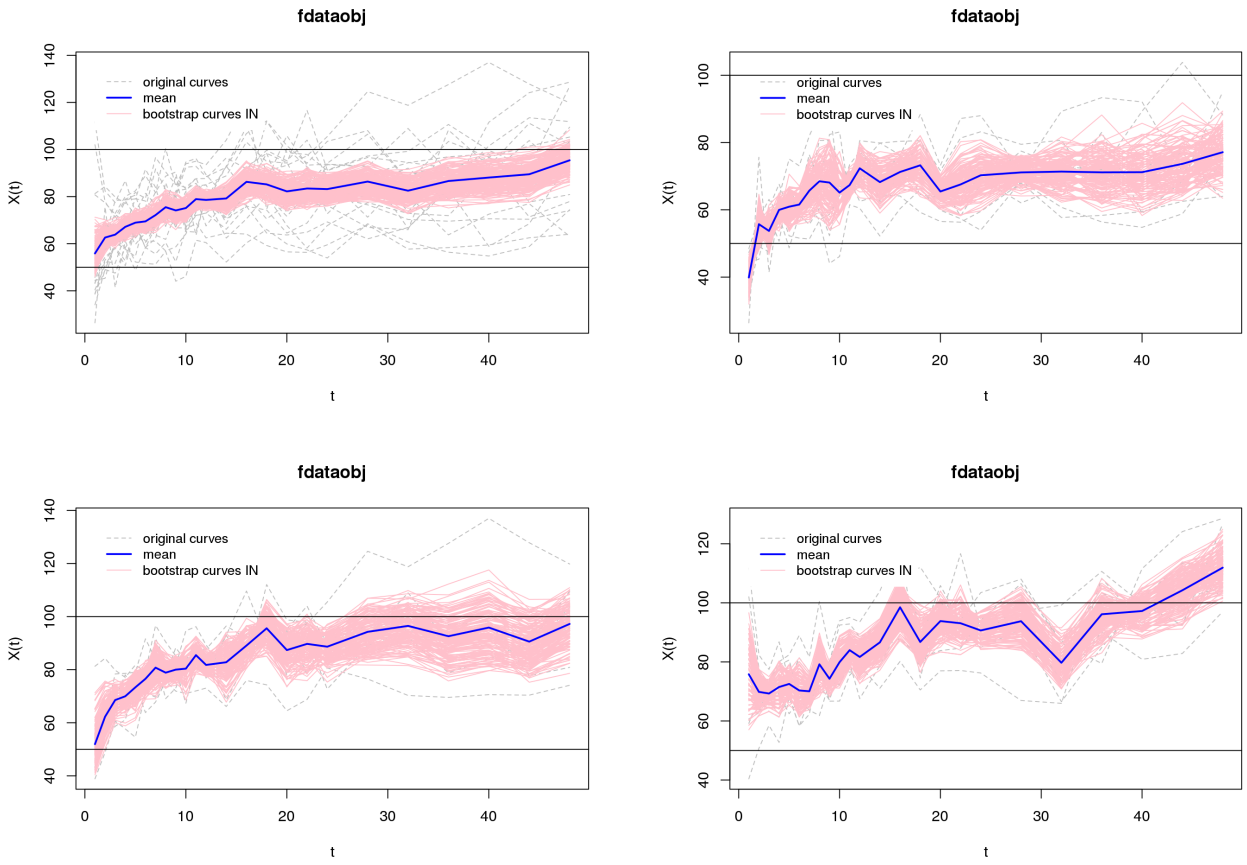

Figure 4: Bootstrap confidence regions for the mean functions: using all data (top-left) and the low (top-right) medium (bottom-left) and high (bottom-right) pump-flows.

tor is considered. A 95% confidence regions for these mean functions are displayed too in figure 4.

5. Conclusions and further developments

In the last decade there has been a significant change in the nature of the active pharmaceutical ingredients (APIs) that formulators have had to deal with. This has led innovator pharmaceutical companies to re-examine methods to deliver compounds other than by the standard, resulting in a renewed interest in use of dry powder inhalers (DPIs) that use hard capsules as the dose containers. Pulmonary drug delivery offers significant and unique

benefits. Inhalers have been used for many years to deliver drug to the lungs to treat airway diseases such as asthma, COPD and more recently, Parkinson, diabetes or cystic fibrosis. The key point in inhalation delivery is the drug particle size, to reach the peripheral airways, where the drug is most efficiently absorbed, particles need to be in the 1-5 μm aerodynamic diameter range. Particles larger than 5 μm usually deposit in the oral cavity or pharynx, from which they are easily cleared. In contrast, particles smaller than 0,5 μm may not deposit at all, since they move by Brownian motion and settle very slowly. The capsule manufacturing process requires the use of an internal surface lubricant on the mold pins on which the capsules are formed. It enables the dried capsule parts (caps and bodies) to be removed from the molds without damage. The quantity of lubricant that remains in the capsule will modify the capsules surface properties and play a key role in capsule aersolization properties (emitted dose and particle size). The sensitivity of the ILC on drug performance is very high. 30 ppm (parts per million) difference may double the performance for some drugs.

In this paper, the internal lubricant content during the whole experimental interval is considered as a whole function i.e. not just values at a given times, so a new approach using functional data analysis have been proposed.

Our analysis has proved that no difference is observed along the time interval when we compare the high and medium pum-flows. A greater difference is observed for the comparisons between low vs medium and high vs low pump-flows. Comparing low and medium pump-flows, we have a significant different (local) mean function from 2 hours up to almost 40. For the comparison between high and low pump-flows a significant different (local) mean function is observed from 0 to one hour, from 12 to 29 hours and from 39 to the end of the time interval, 48 hours.

So the manufacturing parameters that define the capsule ILC have been identified and a model has been proposed in order to make capsules with the internal lubricant content required, so fixing the values of these parameters drug delivery can be controlled. This fact is really significant for designing new drugs or for the generic products development.

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