Quality improvement of melt extruded laminar systems using mixture design

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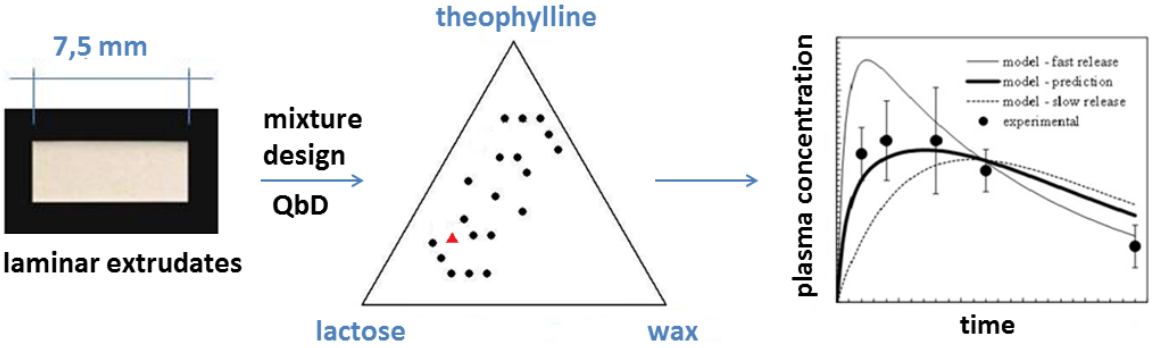

Abstract

This study investigates the application of melt extrusion for the development of an oral retard formulation with a precise drug release over time. Since adjusting the formulation appears to be of the utmost importance in achieving the desired drug release patterns, different formulations of laminar extrudates were prepared according to the principles of Experimental Design, using a design for mixtures to assess the influence of formulation composition on the *in vitro* drug release from the extrudates after 1 h and after 8 h. The effect of each component on the two response variables was also studied.

Ternary mixtures of theophylline (model drug), monohydrate lactose and microcrystalline wax (as thermoplastic binder) were extruded in a lab scale vertical ram extruder in absence of solvents at a temperature below the melting point of the binder (so that the crystalline state of the drug could be maintained), through a rectangular die to obtain suitable laminar systems.

Thanks to the desirability approach and a reliability study for ensuring the quality of the formulation, a very restricted optimal zone was defined within the experimental domain. Among the mixture components, the variation of microcrystalline wax content played the most significant role in overall influence on the *in vitro* drug release. The formulation theophylline:lactose:wax, 57:14:29 (by weight), selected based on the desirability zone, was subsequently used for *in vivo* studies. The plasma profile, obtained after oral administration of the laminar extruded system in hard gelatine capsules, revealed the typical trend of an oral retard formulation.

The application of the mixture experimental design associated to a desirability function permitted to optimize the extruded system and to determine the composition space that ensures final product quality.

Key words:

Laminar extrudates; Sustained release; Mixture Experimental Design; Desirability function; Design Space; *In vivo* studies.

1. Introduction

Melt extrusion is a relatively recent solvent-free pharmaceutical processing technique applicable to both immediate release and sustained release dosage forms (Breitenbach, 2002; Douroumis , 2012). During the process, the mixture of thermoplastic binder, excipients and APIs are fed into the heated barrel, and extruded through the die attached at the end of the barrel (McGinity et al. 2007). The physical shape of the final product depends on the geometrical design of the die, obtaining e.g. cylinders, pipes, laminates, helices or films. In literature, both hollow and solid cylinders are the most studied shape of extrudates (Mehuys et al., 2004; Michalk et al. 2008), even though several authors have investigated the opportunity to change the shape (and hence the specific surface area) to tailor the attributes of the final system (e.g. drug release). Conversely laminar extrudates, being the most "obvious" shape and easiest to produce have been poorly investigated. To our best knowledge, rectangular dies are largely employed in the production of films but the only example of laminar extrudates reported in the literature is that of Pinto and coworkers (Oliveira et al., 2013). These authors have recently successfully produced such kind of extrudates using lipid-based excipients as carriers in absence of solvents and at ambient temperature. Yet, laminar extrudates have a great potential due to their high versatility being suitable for oral, buccal or topical administration. Furthermore, extrudates can be easily cut in different sizes allowing the convenient

adjustment of the drug dose. Finally, this shape is suitable as a final dosage form, as well as to be filled in hard gelatine capsules (Müllers et al., 2013).

The aim of the present investigation is to produce, in a lab scale vertical ram extruder, thin laminar extrudates (7.5x0.5x5 mm) with a targeted *in vitro* release of theophylline (model drug), ranging from 35% after 1 h and 75% after 8 h (Table 1), with the final purpose of producing a retard formulation for oral administration. The choice of thin laminar extrudates was also based on previous studies dealing with the relationship among surface area and drug release in 3 different helical shapes of extrudates (having 2, 3 and 4 blades) (Hasa et al. 2011), and previous experiences with bi-layered cylindrical co-extrudate (Quintavalle et al., 2008). In this case, in order to ensure a successful formulation development, the design of experiments (DoE) principles were adopted to explore the composition space of the formulation. DoE is one of the most important area in drug development. Design and analysis of experiments are considered useful tools for really optimizing the process performance, identifying interactions among process variables, and reducing the process variation that can affect the quality of the output. DoE methodology can be applied in process development and improvement: screening DoE may be used for process parameter screening and statistical DoE for process parameter range determination.

In particular, in this study to better ensure the desired product quality, the influence of formulation factors was analysed by developing quantitative regression models. Being the examined factors ingredients of a formulation, their amount cannot be varied independently, since their proportions sum up to 100%. Thus an experimental design for mixtures (Voinovich et al., 2009) was employed. This strategy has been previously applied to the preparation of hot melt extruded systems by Rambali et al., (2003) and recently by Djuris et al. (2013). To go into more details, several ternary mixtures (composed of theophylline, lactose and wax) according to an experimental design for mixtures were prepared and subjected to melt extrusion, and the influence of formulation composition on the *in vitro* drug release from such extrudates (after 1 h, experimental response Y_1 and after 8 h, Y_2) was estimated. The experimental design for mixtures was hence associated to a

desirability function, to optimize the extruded system. Inside the composition zone defined by desirability function one formulation was selected for *in vivo* studies and hence administered orally in hard gelatine capsules to healthy volunteers. Finally, the *in vivo* performance was studied by performing the pharmacokinetic analysis.

2. Materials and methods

2.1. Materials

Anhydrous theophylline (theo), with a particle mean diameter (±S.D.) of 56 (±18) μm, was obtained from Polichimica s.r.l. (Bologna, Italy). Monohydrate lactose Flowlac®100-Meggle (lac) (having the following particle size distribution: <32 μm ≤ 10%, <100 μm ≤ 20-45%, <200 μm ≤ 80%) was a gift from Faravelli (Milan, Italy). Microcrystalline wax (or paraffinic wax, complex blends of mineral hydrocarbon waxes marketed as Paracera P, wax), with a particle mean diameter (±S.D.) of 204 (±95) μm and a melting range of 58-62°C, was kindly donated by Paramelt (Heerhugowaard, Netherlands). Solvents of HPLC grade were purchased from Carlo Erba (Milan, Italy).

2.2. Preparation of laminar extrudates

The extrudates were prepared using a vertical lab scale ram extruder (Thalassia®, Trieste, Italy) described into details in a previous work (Grassi et al., 2003). Briefly, the movement of the stainless steel ram in this extruder is promoted by an oleodynamic cylinder driven by an electric pump (max pressure 150 bar). The cylindrical nickel plated brass barrel has a capacity of 66 cm^3 and an internal diameter of 25 mm. The die attached at the end of the barrel can be changed on demand: in this case a rectangular die (with a flat entry, 0.5 mm x 5 mm cross section) was used. The barrel, acting as a powder reservoir, can be thermo-stated with an electrically heated jacket (max temp. ~ 120°C). In this case, based on previous experiences with analogous mixtures (Hasa et al., 2011), a temperature of 50°C was chosen.

Prior to extrusion, the powdered materials (ternary mixtures of theo, lac and wax in different proportions according to Table 2) were mixed in 2.81 high shear mixer (Rotolab, Zanchetta®,

Lucca, Italy) for 10 min at 120 rpm. Then, batches of 50 g of each mixture were packed to a constant volume into the barrel, by applying hand pressure, and equilibrated for 1 h at 50°C. Then, the mass was extruded through the rectangular die using a constant pressure. The laminar extrudates were collected and, after cooling at ambient temperature, were sliced up manually by use of a hot cutter in unities of length (7.5 mm) suitable to have a drug content for dissolution studies in sink conditions.

At the end of the extrusion procedure, the samples were subjected to X-Ray Powder diffraction analysis in comparison to pure compounds and physical mixtures prepared in the same weight ratios to check the drug solid state in the samples. These data (not shown) attested that the drug still retains its crystalline state after extrusion, probably thanks to the high content of drug in the laminar extrudates and to the low extrusion temperature, below the melting point of the wax; thus the drug is neither amorphyzed nor molecularly dispersed into the carrier. The presence of the crystalline form of the active principle in the extrudates is in agreement with previous results (Hasa et al., 2011).

2.3. Mixture experimental design

Since the aim of the study was to analyze the influence of formulation composition on the *in vitro* drug release from the laminar extrudates, an experimental design approach for mixtures was applied for the planning of the extrusion experiments.

On the basis of the preliminary trials, the component constraints were set for the percent amount of theophylline $55 \leq x_1 \leq 65$, lactose $5 \leq x_2 \leq 15$, and microcrystalline wax $28 \leq x_3 \leq 32$ to ensure that the experimental composition space contained feasible formulations with our operating conditions (e.g. laminar extrudates with a uniform surface and shape) and to obtain, at the same time, an extrudate with a probable *in vitro* sustained drug release. Due to the aim of the research, the response variables were the *in vitro* drug release from the prepared extrudates after 1 h (Y_1) and after 8 h (Y_2) tested at the conditions reported in the following paragraph 2.6.

Table 1 summarizes the design of the optimization study and acceptance criteria for the drug release.

The experimental plan (shown in Table 2 and Fig. 1), designed using NEMRODW software (Mathieu et al., 2002), consisted of 13 experimental runs (repeated in doubled experiments) and 6 checkpoints. To reduce systematic errors experiments were completely randomized.

2.4. Data analysis and validation of the optimisation model

The special cubic model proposed by Scheffé (Scheffé, 1958; Lewis et al., 1999) was postulated to derive relationships between the responses (*in vitro* drug release at 1h and 8h) and mixture compositions, and to select an optimal extrudate formulation (Eq. 1)

$$\eta = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_{12} + \beta_{13} X_{13} + \beta_{23} X_{23} + \beta_{123} X_{123} \quad (1)$$

According to the steps involved in the response surface methodology (RSM) (Voinovich et al., 2009), after performing the mixture experiment by running every treatment combination given by the design and checkpoints, the coefficients of the experimental model were firstly estimated on the experimental data obtained by omitting the checkpoints. In this way, it was possible to validate the model by comparing the experimental data collected at the checkpoints with the corresponding response values calculated by the model.

2.5. Robust optimization and reliability study

In order to ensure a robust optimization, the desirability function approach (Derringer and Suich, 1980) was also used. The desirability approach is one of the most applied methods whenever a compromise that best satisfies a set of predetermined objectives is searched for. The desirability function approach allows finding operating conditions for an optimal solution in two steps:

1) transformation of every response as a function of the objectives under the form of an elementary desirability function (d_j)

$$d_j = T_j(Y_j, objectives) \quad (2)$$

2) creation of a global desirability function (D) characterizing the global compromise given by the geometric mean of the desirabilities

$$D = \left[\prod_{i=1}^{r} d_i \right]^{1/r} \quad (3)$$

The desirability function estimates a percentage of compromise satisfaction. If a criterion value is not acceptable, the global desirability function is null, thus the compromise is rejected. Thanks to these transformations, at every point of the domain, the global desirability function can be calculated.

Once the value of the responses at every point of the region of interest is determined (in validated models), every estimated response ($Y_{cal,i}$) can be transformed to a value d_i representing the acceptance percentage of the value $Y_{cal,i}$ in relation to the fixed objectives (or desires) for the considered response. The calculation methods proposed for this transformation permit to obtain a percentage d_i so that $0\% \leq d_i \leq 100\%$. If $d_i = 100\%$, the response Y_1 calculated at this point is equal to its optimal value (called target value). On the contrary, if $d_i = 0\%$, the response Y_1 calculated at this point is over the tolerance limits.

When the optimum of the *D* function is known, the composition that permits to best satisfy the constraints and the wideness of the optimal zone (corresponding to the space where the *D* function is possible) are determined. However, complementary information is required. It is necessary to know, inside the experimental domain of interest, what is the zone where the estimation of all the responses can be calculated with a probability $\leq \alpha$ % of not respecting the fixed constraints for every response.

2.6. Assay of drug content

Samples of laminar extrudates were gently milled in a mortar with a pestle and dispersed in absolute ethanol and filtered (0.2 μm, PES, Millipore Millex-HA). The concentrations of theophylline in the samples were measured using a previously described HPLC method (Voinovich et al., 2000). The percent drug content was compared to the calculated value. The experimental values were the mean values of three replicates.

2.7. *In vitro* release

In *vitro* dissolution tests (conducted in triplicates) were performed under sink conditions ($c \leq 0.2\ c_s$) using the USP rotating basket apparatus (Pharmatest, Steinheim, Germany), with a stirring rate of 100 rpm, in 900 ml of purified water containing 0.1% polysorbate 20, thermostated at 37 ± 0.5°C. Slices of extrudates corresponding to 25 mg of theophylline were tested. The solution was filtered and continuously pumped (12.5 ml/min) to a flow cell in a spectrophotometer (UV2 Spectrometer, Unicam, Madison, WI). The amount of drug dissolved was analyzed at the maximum absorbance wavelength of the drug (271 nm). The results were reported as the mean values of three-replicated experiments and standard deviations were within 5% of the mean value.

2.8. In vivo Studies

For this experiment four healthy volunteers, aged between 25-55 years, and weighing on average 70 kg were chosen. Before the analysis a written informed consent was signed by each subject. The research followed the tenets of the Declaration of Helsinki promulgated in 1964 and was approved by the institutional human experimentation committee. All the volunteers had normal hepatic and renal function. All subjects did not take any drug before and fasted from 12 h before drug administration until lunch on the treatment day. They were also asked not to take coffee or alcoholic beverages, nor smoking 12 h before and 24 h after the drug administration. The subjects were all given a standard lunch 6 h after the dosing, while were allowed to drink water during the treatment period.

The dose (400 mg) of theophylline (corresponding to 16 slices of 7.5mm) was administered in 2 type 00 hard gelatine capsules. Blood samples (5 ml) were drawn at 2, 4, 8, 12 and 24 h following capsule administration. Each sample was collected in a heparinised tube, and plasma was immediately separated by centrifugation (at 5000 g for 15 min), subsequently frozen and stored at -20°C until assayed.

2.9. Sample analysis

The concentrations of theophylline in the plasma samples were measured using a previously described HPLC method (Voinovich et al., 2000).

2.10. Pharmacokinetic analysis

Individual plasma concentration-time profiles were analyzed by non-compartmental pharmacokinetic analysis using WinNonlin version 2.1 (Pharsight, Mountain View, CA, USA). Maximum plasma concentration (C_{max}) and corresponding sampling time (t_{max}) were recorded as observed. Half-life of the terminal phase ($t_{1/2,z}$) was calculated as the natural logarithm of 2 divided by the slope (λ_z) of the linear regression line of the terminal log-linear portion of the concentration-time curve. Area under the plasma concentration curve from time 0 to the last measured concentration at 24 h (AUC_{24}) was calculated by the linear trapezoidal rule and extrapolated to infinite time (AUC_{∞}) by addition of the term $C(24)/\lambda_z$, where C(24) is theophylline plasma concentration at 24 h. Mean residence time (MRT) was calculated as AUMC/AUC, where AUMC is the area under the first moment curve.

2.11. *In vitro/ in vivo* mathematical modelling

The *in vitro* and the *in vivo* data were analysed by a previously presented mathematical model that relies on the following assumptions (Hasa et al., 2011):

1) drug pharmacokinetics can be represented by a two-compartments model with first order elimination in the blood, 2) drug release kinetics in the G.I. fluids is not affected by the absorption, metabolism and gastrointestinal transit and it can be replaced by the *in vitro* release kinetics and 3) the drug concentration in the central compartment (blood plasma) (C_c) can be always retained negligible in comparison to the drug concentration in the gastrointestinal fluids (C_{GI}). Accordingly, model equations read:

$$\frac{dC_{GI}}{dt} \approx R(t) - k_{ab}\frac{V_c}{V_{GI}}C_{GI} \qquad k_{ab} = \frac{AP}{V_c} \tag{4}$$

$$\frac{dC_c}{dt} = k_{ab}C_{GI} - (k_{el} + k_{cp})C_c + \frac{k_{pc}}{f}C_p \qquad f = \frac{V_c}{V_p} \tag{5}$$

$$\frac{dC_p}{dt} = k_{cp} f\, C_c - k_{pc}C_p \tag{6}$$

where t is time, $R(t)$ represents the drug release kinetics coincident with that measured *in vitro*; V_c and V_{GI} are the volumes of the central (blood plasma) and gastrointestinal G.I. compartments, respectively; k_{el} and k_{ab} are, respectively, the elimination and the absorption rate constants; A is the G.I. tract geometrical surface (i.e. that not accounting for the presence of villi and microvilli that is considerably higher); P is G.I. apparent permeability, k_{cp} and k_{pc} are distribution rate constants from the central to the peripheral compartment, respectively; f is the ratio between volumes of the central (V_c) and peripheral (V_p) compartments; and C_p indicates drug concentration in the peripheral compartment (tissue).

Eqs. (4)-(6) differ from the usual expression of pharmacokinetic equations (Holz and Fahr, 2001) for the assumption that drug distribution among blood and tissues is ruled by drug concentration instead of by drug mass. This leads to the introduction of parameter f that renders the model more physiologically oriented as it allows differentiating the extension of blood and tissue volumes. It is worth noticing that when $f = 1$, this approach and the traditional one (Holz and Fahr, 2001) coincide. In addition, when k_{cp} is set to zero, Eqs. (4)-(6) represent the simpler one compartment pharmacokinetics model. $R(t)$ is determined assuming that *in vitro* release kinetics can be properly fitted by a sum of m exponential functions:

$$\frac{M_t}{M_\infty} = 1 - \sum_{i=1}^{i=m} A_i e^{-k_i t} \qquad \sum_{i=1}^{i=m} A_i = 1 \tag{7}$$

$$R(t) = \frac{M_0}{V_r}\frac{d}{dt}\left(1 - \sum_{i=1}^{i=m} A_i e^{-k_i t}\right) = C_{r0}\sum_{i=1}^{i=m} A_i k_i e^{-k_i t} \tag{8}$$

where m, A_i and k_i are parameters to be determined by fitting eq.(7) to the *in vitro* release data and M_0 is the drug dose. Insertion of eq.(8) into eq.(4) assuming that $C_{GI} = C_c = C_p = 0$ when $t = 0$, leads to model analytical solutions:

$$C_c = C_{r0} k_{ab} \left[G_1 e^{z_1 t} + G_2 e^{z_2 t} + G_3 e^{z_3 t} + \sum_{i=1}^{i=m} \frac{A_i k_i}{\alpha - k_i} \left(\frac{k_{pc} f - k_i}{(k_i + z_1)(k_i + z_2)} \right) e^{-k_i t} \right] \tag{9}$$

$$G_1 = \sum_{i=1}^{i=m} \frac{A_i k_i}{(\alpha - k_i)(z_1 - z_2)} \left[\frac{\alpha - k_{pc} f}{(\alpha + z_1)} + \frac{k_{pc} f - k_i}{(k_i + z_1)} \right] \tag{10}$$

$$G_2 = \sum_{i=1}^{i=m} \frac{A_i k_i}{(\alpha - k_i)(z_2 - z_1)} \left[\frac{\alpha - k_{pc} f}{(\alpha + z_2)} + \frac{k_{pc} f - k_i}{(k_i + z_2)} \right] \tag{11}$$

$$G_3 = \sum_{i=1}^{i=m} \frac{A_i k_i}{(\alpha - k_i)} \frac{\alpha - k_{pc} f}{(\alpha + z_2)(\alpha + z_1)} \tag{12}$$

$$\alpha = k_{ab} \frac{V_c}{V_{GI}} \qquad p = k_{el} + k_{cp} + f\, k_{pc} \qquad q = k_{el} k_{pc} f \qquad z_{1,2} = \frac{-p \pm \sqrt{p^2 - 4q}}{2} \tag{13}$$

3. Results and Discussion

In this optimization study a mixture experimental design in a constrained region of interest was used to estimate the model coefficients in Eq. (1) in order to represent and analyze the response surfaces obtained on the basis of the experimental trials on the formulations. The experimental results (expressed as mean values) along with the standard deviations (SD) are reported in Table 2.

The special cubic models fitted to the experimental data (checkpoints included after model validation) along with the adjusted R^2 are given in Table 3. It is worth of note that the calculated responses, y_1 and y_2 are expressed in terms of the percentages of the pseudocomponents X_i.

For the comparison of the two response surfaces reported in Table 3, the corresponding contour plots are shown in Fig. 2.

In order to attain a robust optimization for the drug release, it was required to be very close to a desired value - the target value - in this case 35% for the release after 1 hour and 75% for the

release after 8 hours (Fig. 3). A desirability function approach was therefore used. For this desirability study, the objectives were expressed by the following limits for the two responses here considered:

$$30\% \leq Y_1 \leq 40\%$$

$$70\% \leq Y_2 \leq 80\% \quad (15)$$

The best solution identified for the formulation is given in Table 4 along with desirability functions for the optimisation, d_i ($\hat{Y}_i$) the partial desirability and the D the overall desirability. The optimal experimental conditions are expressed both in terms of pseudocomponents X_i and as original components. In Fig. 4 (top) the variation of the desirability function is graphically represented.

For a robust optimization the desirability function was further assessed and optimized in terms of reliability. The purpose was to identify the zone where the estimation of all responses can be calculated with a probability ≤ α % of not respecting the fixed constraints for every response. For the two responses under study it was imposed that:

$$\text{Prob}[(30\% < Y_1 < 40\%] \geq 0.80$$

$$\text{Prob}[(70\% < Y_2 < 80\%] \geq 0.80. \quad (16)$$

Thanks to the respect of the constraints with a probability ≥ (1- α), the optimal zone was reduced with the distinction of two limited areas (Fig. 4, bottom). These two areas were further analysed in order to define the composition space that ensures the quality of the final product. The allowable operational flexibility in the ternary formulation (theophylline:lactose:wax) for the two areas are summarized in Table 5.

In Fig. 5 the composition spaces (delimited by the black triangles) are displayed along with the tested formulations with the mixture design (black circles).

While the A area was very small and in proximity to the lower boundary, inside the B area the formulation theophylline:lactose:wax, 57:14:29 (%) was selected to be used for the *in vivo* studies. This formulation guaranteed an *in vitro* drug release of 33% after 1h (Y_1), and of 76% after 8h (Y_2) (Fig. 6).

The *in vitro* performance of this formulation was also analysed by a previously presented mathematical model (Hasa et al., 2011). Release kinetics model Eq.(8) was fitted to the observed *in vitro* release data (Fig. 6). The model was in very good agreement with the observed data ($F(2, 177, 0.95) < 16903$) and the estimated parameters (A_1, k_1, k_2) values were $A_1 = (0.66 \pm 0.003)$, $k_1 = (0.128 \pm 0.001)$ h^{-1}, $A_2 = 1 - A_1$ and $k_2 = (1.23 \pm 0.05)$ h^{-1}. Accordingly, *in vitro* release kinetics could be described by a slow (A_1, k_1) and a fast (A_2, k_2) component being the slow component the prevailing one. Fig. 6 shows the *in vitro* release kinetics predicted by Eq.(8) in the hypothesis that only the slow (dotted line; $A_1 = 1$, $A_2 = 0$, $k_1 = 0.128$ h^{-1}) or only the fast (solid thick line; $A_1 = 0$, $A_2 = 1$, $k_2 = 1.23$ h^{-1}) component rules the release kinetics. In the first case, the fractional drug release ($100*M_t/M_\infty$) is not complete within the experimental time (500 min) while, in the second case, the release process is almost complete after 200 minutes.

Bioavailability parameters are reported in Table 6. These results demonstrate that the selected formulation behaves as a sustained release formulation. Comparison of the observed AUC value with the AUC value of the commercial sustained release formulation of theophylline (Henrist et al., 1999) indicates a trend of improved bioavailability. The observed values of $t_{1/2,z}$ were higher than the reported theophylline elimination half-life of 6 to 10 h (Jonkman et al., 1989), indicating a flip-flop phenomenon. The rate of absorption was therefore manifested in the terminal slope of the concentration-time curve. In vivo behaviour of the tested formulation was similar to the previously evaluated experimental sustained release cluster matrix tablets containing hydrophobic wax and hydrophilic polymer granules (Hayashi et al., 2007).

Parameters of the *in vitro* release model Eq.(8) (A_1, k_1, A_2, k_2) and theophylline pharmacokinetics parameters determined elsewhere (Hasa et al., 2011) ($V_c = 19.3$ l, $f = 1$, $k_{el} = 0.12$ h-1, $k_{ab} = 0.13$ h-1, $k_{cp} = 2.7$ h^{-1}, $k_{pc} = 2.9$ h^{-1}, $M_0 = 400$ mg, $V_r = 300$ ml), were used for simulation of concentration. The simulation of Eq.(10) was in good agreement with the experimental theophylline plasma concentration-time course as shown in Fig. 7. Indeed, this prediction falls within the standard deviation associated with the concentration data up to 12 h, while there is minor discrepancy with

the final concentration measurement at 24 h. It is interesting to notice (see Fig. 7) the considerable variation of the predicted plasma concentration when only the fast (solid thin line) or only the slow (dotted line) are considered for the description of the release phenomenon (see eq.(5) and (6)). This witnesses the importance played by a proper designing of the release process.

Conclusion

This study attested that laminar melt extrusion is a suitable technique for the production of a novel oral retard formulation with a precise drug release over time. The application of the experimental design for mixtures, associated to a desirability function, permitted to define the robustness of the formulation by defining acceptable quality of the product.

Acknowledgment

Authors are extremely grateful to Roger Phan-Tan-Luu (former Professor at the Aix-Marseille University, Marseille, France) for his precious help and tireless support for this study. They are particularly in debt for his suggestions and comments.

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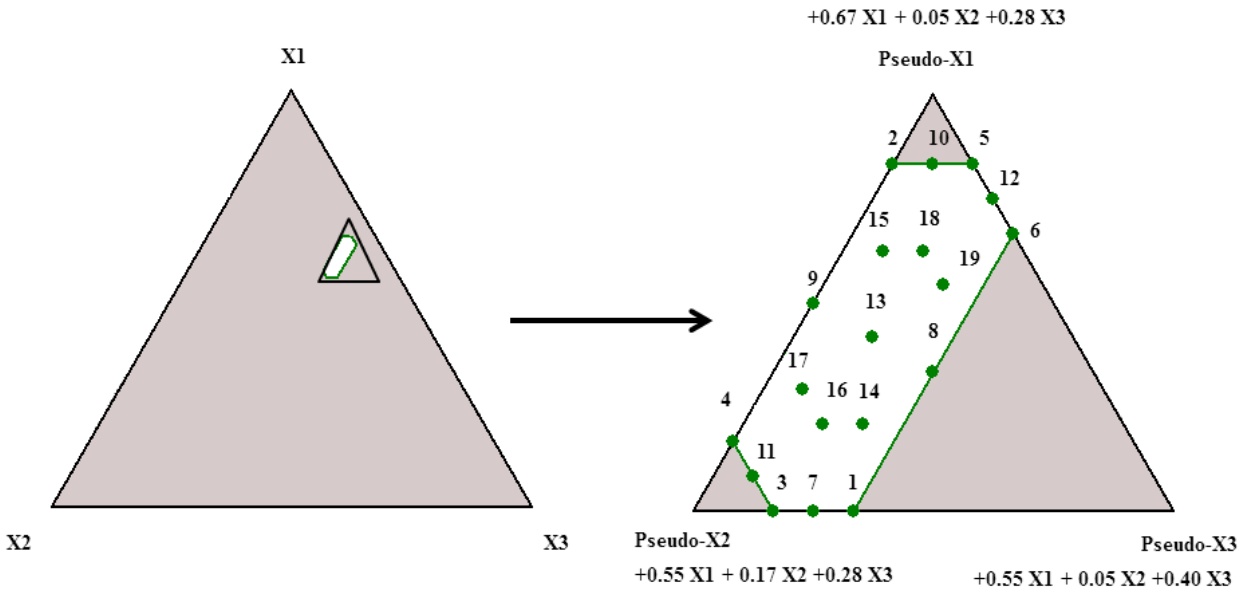
1. Legends for Figures
2. Fig. 1. The constrained experimental region in the three-component system given by the restrictions on the component proportions $0.55 \leq X1 \leq 0.65$, $0.05 \leq X2 \leq 0.15$ and $0.28 \leq X3 \leq 0.32$; black dots represent experimental points.
3. Fig. 2. Contour plot for Y1 (top) and Y2 (bottom) in the constrained experimental region.
4. Fig. 3. Desirability function for the two studied experimental responses: Y1 (top) and Y2 (bottom).
5. Fig. 4. Contour plot of desirability function before (top) and after the reliability study (bottom).
6. Fig. 5. Optimal composition spaces (delimited by black triangles) for the two considered response variables, along with the tested formulations (black circles).
7. Fig. 6. Eq.(7) best fitting (solid thick line) to experimental in vitro release data (open circles; vertical bars indicate standard deviation). Dotted and solid thin line indicate, respectively, eq.(7) prediction about the release kinetics assuming that only the slow (A1 = 1, k1 = 0.128 h-1) or the fast (A2 = 1, k2 = 1.23 h-1) release component rules the drug delivery. t is time while $Mt/M\infty$ indicates the fractional release.

Fig. 7. Comparison between the experimental theophylline plasma concentration (filled circles; vertical bars indicate standard deviation) and eq.(9) prediction (solid thick line) assuming the parameters (A1 = 0.66, k1 = 0.128 h-1, A2 = 0.34, k2 = 1.23 h-1) coming from eq.(7) best fitting to in vitro release data (see Fig. 6) and the theophylline pharmacokinetics parameters elsewhere determined (Hasa et al., 2011) (Vc = 19.3 l, f = 1, kel = 0.12 h-1, kab = 0.13 h-1, kcp = 2.7 h-1, kpc = 2.9 h-1, M0 = 400 mg, VGI = 300 ml). The dotted and the solid thin line indicate, respectively, eq.(9) prediction assuming that only the slow (A1 = 1, k1 = 0.128 h-1) or the fast (A2= 1, k2 = 1.23 h-1) release component rule the drug delivery. t is time while Cc indicates the theophylline plasma concentration.


X1
X2
X3
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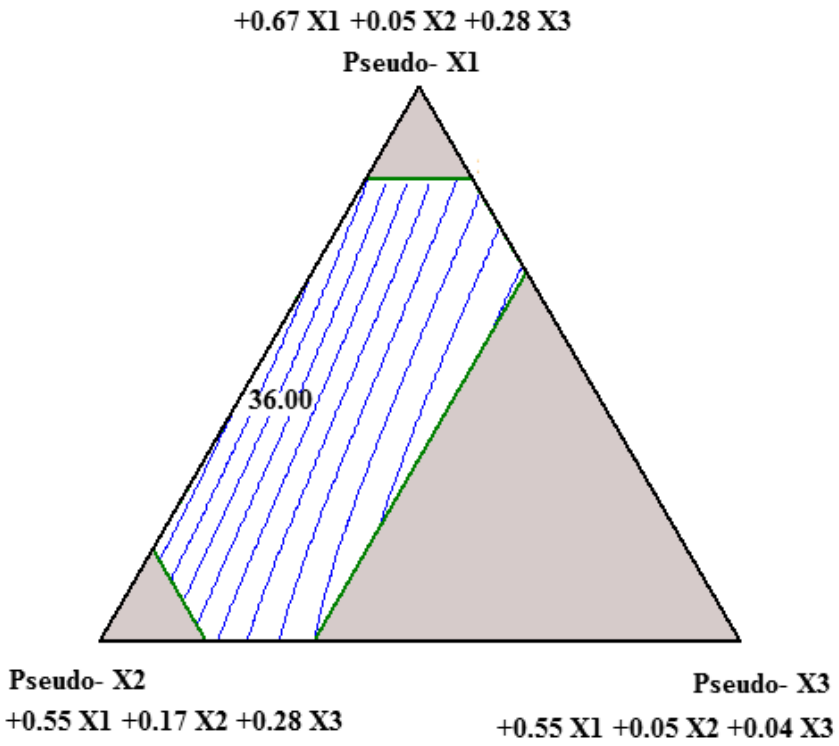
Pseudo-X1
2
10
5
12
6
15
18
19
9
13
8
17
16
14
4
11
3
7
1
Pseudo-X2
+0.55 X1 + 0.17 X2 +0.28 X3
Pseudo-X3
+0.55 X1 + 0.05 X2 +0.40 X3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Pseudo- X1
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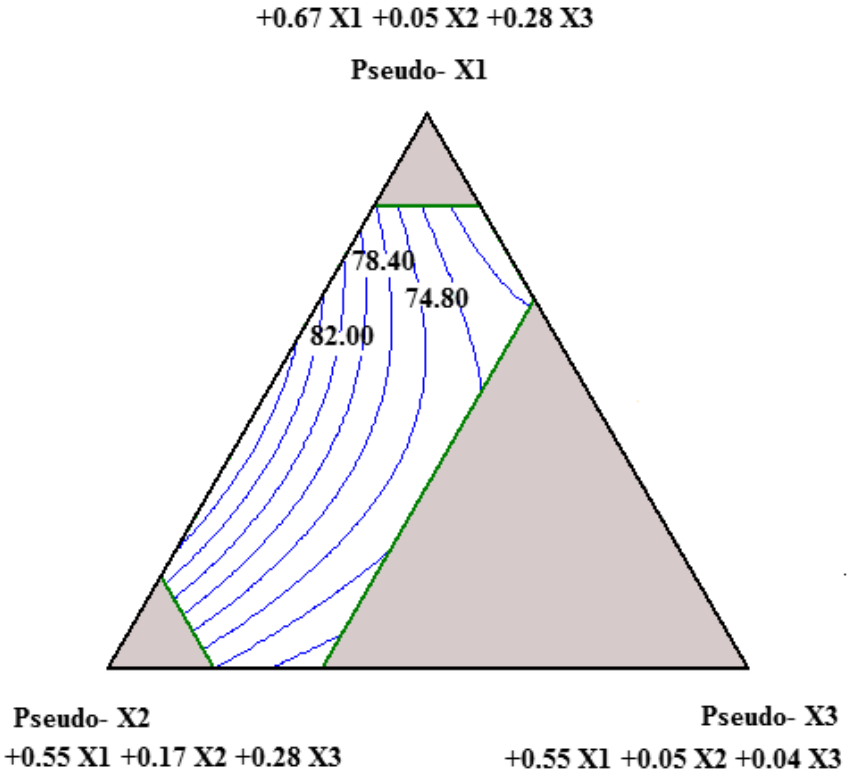
Pseudo- X3
+0.55 X1 +0.05 X2 +0.04 X3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Pseudo- X1
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74.80
82.00
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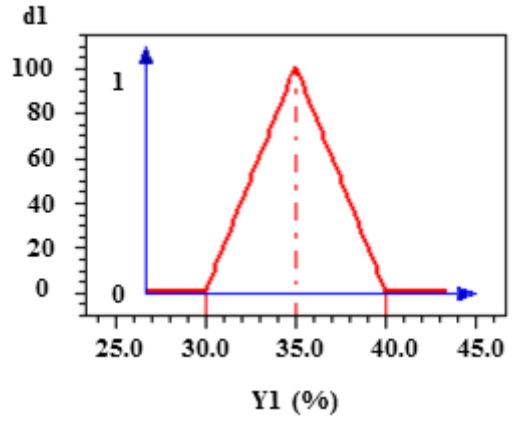
+0.55 X1 +0.17 X2 +0.28 X3
Pseudo- X3
+0.55 X1 +0.05 X2 +0.04 X3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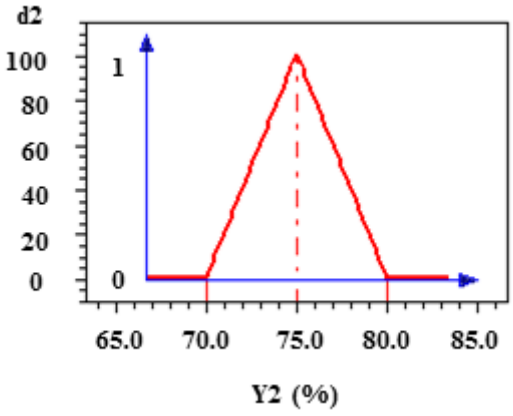
80
60
40
20
0
1
0
25.0
30.0
35.0
40.0
45.0
Y1 (%)


d2
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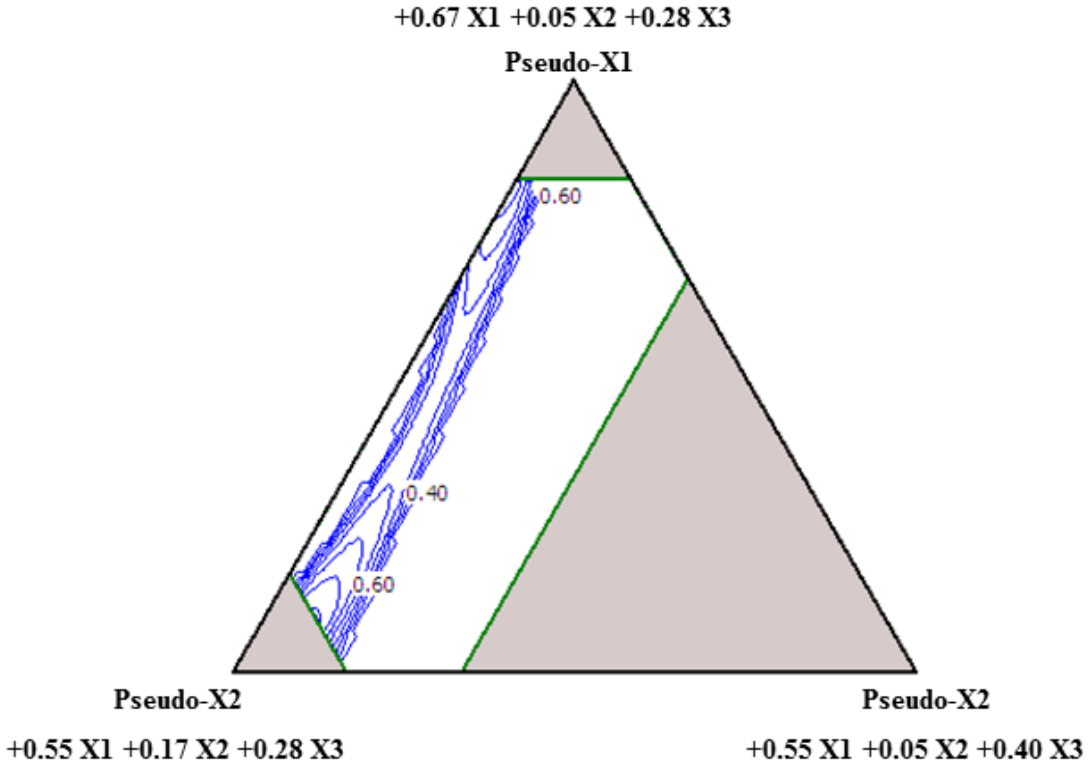
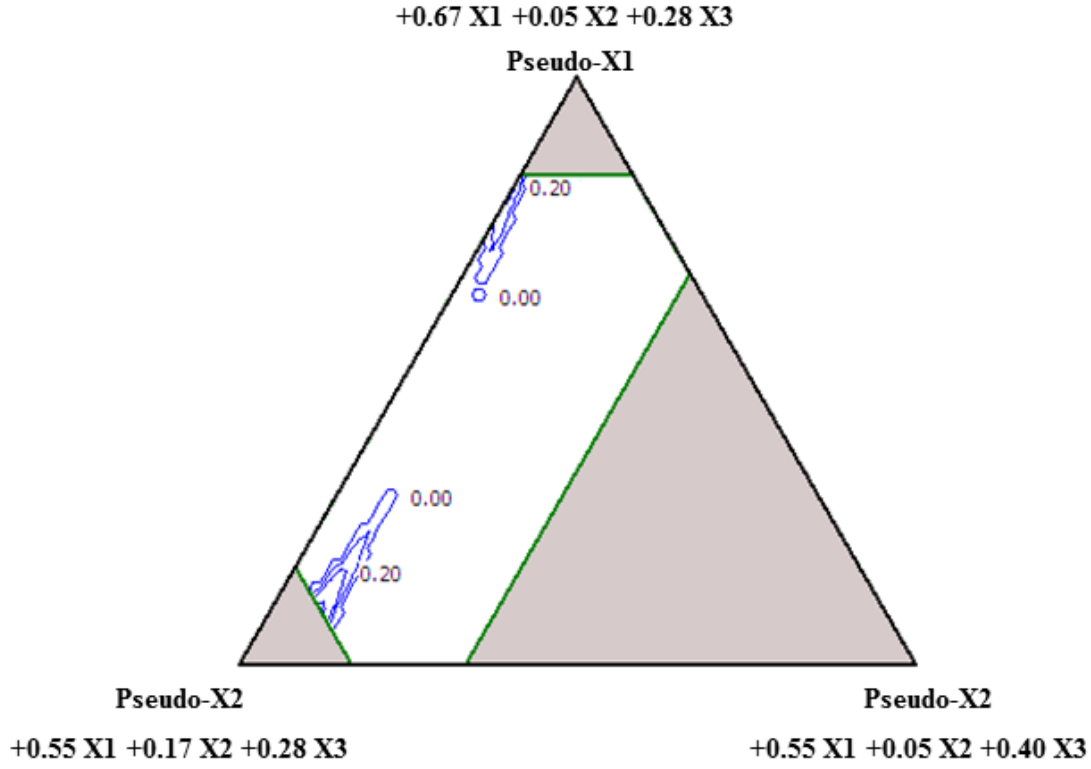
80
60
40
20
0
1
0
65.0
70.0
75.0
80.0
85.0
Y2 (%)


+0.67 X1 +0.05 X2 +0.28 X3
Pseudo-X1
0.60
0.40
0.60
Pseudo-X2
+0.55 X1 +0.17 X2 +0.28 X3
Pseudo-X2
+0.55 X1 +0.05 X2 +0.40 X3


+0.67 X1 +0.05 X2 +0.28 X3
Pseudo-X1
0.20
0.00
0.00
0.20
Pseudo-X2
+0.55 X1 +0.17 X2 +0.28 X3
Pseudo-X2
+0.55 X1 +0.05 X2 +0.40 X3

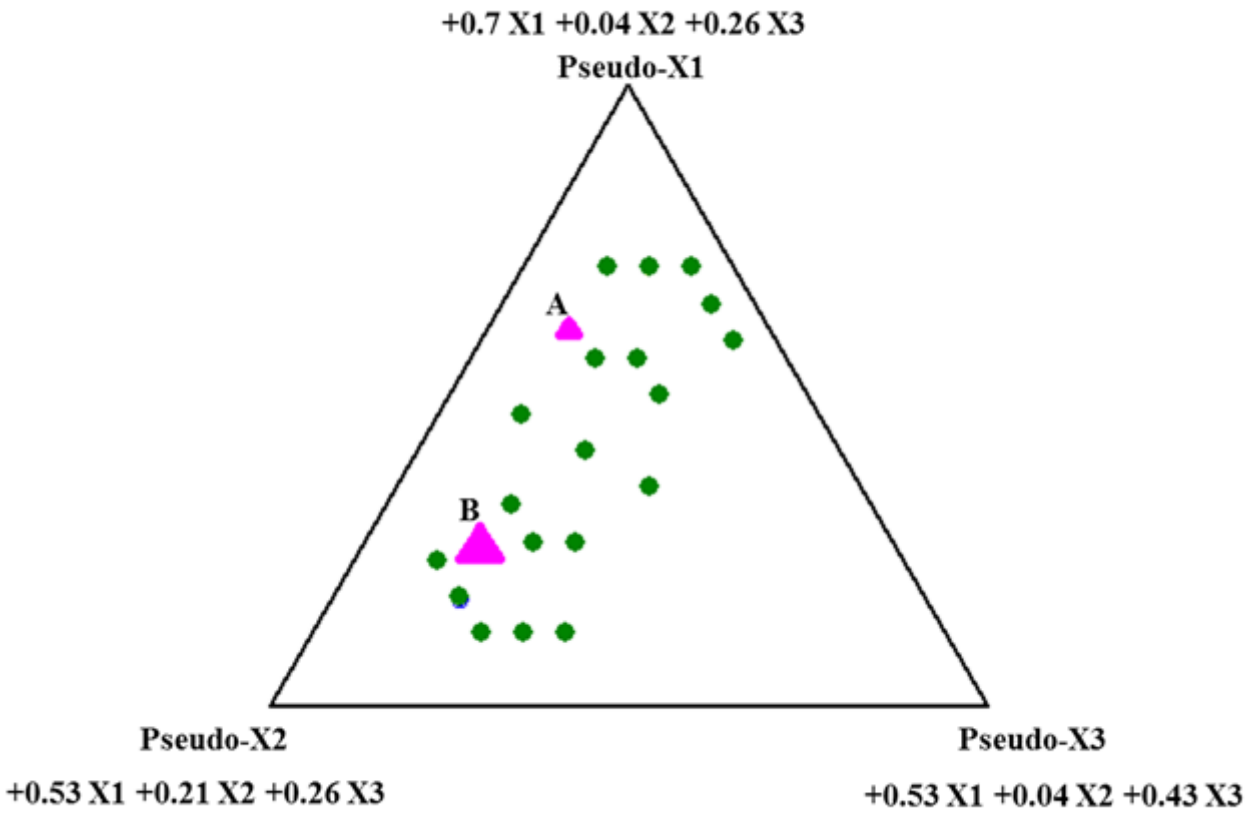

+0.7 X1 +0.04 X2 +0.26 X3
Pseudo-X1
A
B
Pseudo-X2
+0.53 X1 +0.21 X2 +0.26 X3
Pseudo-X3
+0.53 X1 +0.04 X2 +0.43 X3

Table 1. Design of the mixture study for the drug release optimization.

Associated variable	Factors: formulation components	Lower limit %	Upper limit %
x_1	theophylline	55	65
x_2	monohydrate lactose	5	15
x_3	microcrystalline wax	28	32
	Responses	**Target values**	
y_1	Drug release after 1 hour	35%	
y_2	Drug release after 8 hours	75%	

Table 2. Design point coordinates in the constrained region inside the factor space given by three blend components, along with experimental results (two replications for experiments 1 to 13).

	Formulation factor (%)			Experimental response (%)	
Design points	x_1	x_2	x_3	y_1	y_2
1	55.0	13.0	32.0	23.0, 25.0	67.0, 70.0
2	65.0	7.0	28.0	31.0, 33.0	73.0, 78.0
3	55.0	15.0	30.0	27.0, 29.0	74.0, 69.0
4	57.0	15.0	28.0	35.0, 38.0	81.0, 83.0
5	65.0	5.0	30.0	24.0, 21.0	64.0, 67.0
6	63.0	5.0	32.0	20.0, 17.0	72.0, 68.0
7	55.0	14.0	31.0	23.0, 26.0	69.0, 70.0
8	59.0	9.0	32.0	21.0, 17.0	71.0, 70.0
9	61.0	11.0	28.0	34.0, 36.0	84.0, 80.0
10	65.0	6.0	29.0	27.0, 30.0	71.0, 73.0
11	56.0	15.0	29.0	32.0, 37.0	76.0, 73.0
12	64.0	5.0	31.0	21.0, 22.0	68.0, 70.0
13	60.0	10.0	30.0	26.0, 24.0	78.0, 77.0
14*	57.5	11.5	31.0	26.0	70.0
15*	62.5	8.5	29.0	30.0	73.0
16*	57.5	12.5	30.0	26.0	71.0
17*	58.5	12.5	29.0	35.0	75.0
18*	62.5	7.5	30.0	25.0	71.0
19*	61.5	7.5	31.0	23.0	74.0

*checkpoints

Table 3. Response models and statistical data obtained from ANOVA after the model validation step

Response	**Special cubic model**	**Adjusted R^2**	**Significance**
y_1	$30.29X_1+37.55X_2+16.83X_3+6.46X_1X_2-32.39X_1X_3 -35.57X_2X_3-39.28X_1X_2X_3$	0.88	<0.01***
y_2	$69.00X_1+78.03X_2+11.43X_3+34.12X_1X_2-59.96X_1X_3 -92.92X_2X_3-36.91X_1X_2X_3$	0.76	<0.01***

Significance code (α): *** p= 0.001 (0.1%)

Table 4. Optimal experimental conditions and predicted responses

Pseudocompomponents	*Original components*	*Predicted properties*	*Desirability (d_i)*
$X_1 = 0.09$	Theophylline = 0.56	$y_1 = 33$ %	d_1 ($\hat{y}_1$)= 82.52
$X_2 = 0.83$	Lactose = 0.15	$y_2 = 76$%	d_2 ($\hat{y}_2$)= 62.96
$X_3 = 0.08$	Wax = 0.29		D = 72.08

Table 5. Allowable operational flexibility in the ternary formulation (%) for the two areas.

Area A	Area B
63.2 < Theophylline <63.5	57.1 < Theophylline < 57.7
8.3 < Lactose <8.6	13.6 < Lactose <14.2
28.2 < Wax < 28.5	28.7 < Wax < 29.3

Table 6. Bioavailability parameters after oral administration of 400 mg of theophylline as an experimental sustained release formulation to four healthy subjects.

Parameter	Mean (SD)
t_{max} (h)	5.00 (3.46)
C_{max} (mg/l)	6.94 (0.77)
AUC_{24} (mg h/l)	106.5 (13.6)
λ_z (h^{-1})	0.0653 (0.0087)
$t_{1/2,z}$ (h)	10.77 (1.56)
AUC_∞ (mg h/l)	140.3 (27.1)
MRT (h)	17.0 (3.2)