IN VIVO ABSORPTION BEHAVIOUR OF THEOPHYLLINE FROM STARCH-METHYL METHACRYLATE MATRIX TABLETS IN BEAGLE DOGS

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Abstract:

This study evaluates *in vivo* the drug absorption profiles from potato starch-methyl methacrylate matrices* using theophylline as a model drug. Healthy beagle dogs under fasting conditions were used for *in vivo* studies and plasma samples were analyzed by a fluorescence polarization immunoassay analysis (FPIA method). Non-compartmental and compartmental (population approach) analysis was performed to determine the pharmacokinetic parameters. The principle of superposition was applied to predict multiple dose plasma concentrations from experimental single dose data. An *in vitro-in vivo* correlation (IVIVC) was also assessed. The sustained absorption kinetics of theophylline from these formulations was demonstrated by comparison with two commercially available oral sustained-release theophylline products (Theo-Dur® and Theolair®). A one-compartment model with first order kinetics without lag-time best describes the absorption/disposition of theophylline from the formulations. Results revealed a theophylline absorption rate in the order FD-HSMMA ≥ Theo-Dur® ≥ OD-CSMMA > Theolair® ≥ FD-CSMMA. On the basis of simulated plasma theophylline levels, a twice daily dosage (every 12h) with the FD-CSMMA tablets should be recommended. A Level C IVIVC was found between the *in vitro* $t_{50\%}$ and the *in vivo* AUC/D, although further optimization of the *in vitro* dissolution test would be needed to adequately correlate with *in vivo* data.

Key words: Potato starch-methyl methacrylate copolymers; Anhydrous theophylline; Sustained-release matrix tablet; Beagle dog; Pharmacokinetics; IVIVC.

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* FD-HSMMA: freeze-dried hydroxypropylstarch methyl methacrylate; OD-CSMMA: oven-dried carboxymethylstarch methyl methacrylate; FD-CSMMA: freeze-dried carboxymethylstarch methyl methacrylate.

1. Introduction

Theophylline is a methylxanthine derivative widely used for its bronchodilatory, inotropic, central stimulant and diuretic effects both in humans and animals (Mengozzi et al., 1998). A very close relationship has been reported between plasma drug concentrations and its efficacy/safety. The main limitation to therapeutic effectiveness is its low therapeutic index. Therapeutic plasma concentrations are ranging from 5 to 15 μg/mL and plasma concentrations greater than 20 μg/mL may result in adverse effects (Muskó et al., 2001). After oral administration as a solution, theophylline is rapidly and completely absorbed. A single dose of 5 mg/kg in adults provides a mean peak serum concentration of ≈ 10 mcg/mL (range 5-15 mcg/mL) at 1-2 hr after the dose (FDA, 2012). This rapid absorption leads to frequent administration to maintain therapeutic drug levels. Sustained-release formulations are desired to maintain plasma concentrations within the therapeutic range during a more lasting period of time, avoiding adverse effects and leading to improve efficacy and to better patient compliance.

The most common method of modulating the drug release is to develop polymeric matrix tablets. In this type of systems, judicious selection of release retarding excipients is necessary. Over the last years, a new generation of grafted copolymers combining potato starch derivatives (hydroxypropylstarch -HS- or carboxymethylstarch -CS-) with an acrylic monomer (methyl methacrylate -MMA-) were introduced as matrix-forming excipients for oral sustained-release dosage forms. As described elsewhere (Castellano et al., 1997), these copolymers were synthesised by free radical polymerisation of the monomer (MMA) on the starches using Ce (IV) as an initiator. The products obtained (HSMMA, CSMMA) were either dried in a vacuum oven at 6.67-13.33 hPa and 50 ºC until constant weight (OD copolymers) or freeze-dried (freezing process at -20 ºC for 24h and sublimation process at

0.13 hPa and -50ºC until a powdered product was obtained) (FD copolymers). These materials were thoroughly characterized in terms of physico-chemical and technological properties (Bravo-Osuna et al., 2005; Ferrero et al., 1999; Ferrero and Jiménez-Castellanos, 2002) and demonstrated their ability to form inert matrix tablets that control drug release by a diffusion mechanism (Ferrero et al., 2003).

Several studies have been reported focused on the influence of excipients and technology on bioavailability of sustained release theophylline formulations (Ikegami et al., 2006; Miyazaki et al., 2000, 2001; Roshdy et al., 2002; Yu et al., 1996). It is also known that for oral sustained-release dosage forms the release rate is the limiting factor in the absorption process. Therefore it is desirable to use the *in vitro* data to predict *in vivo* pharmacokinetic parameters for a rational development and evaluation process of these sustained-release dosage forms. Thus over the past decade, interest has increased on *in vitro-in vivo* correlations (IVIVC) (FDA, 1997). In this way, as theophylline is a Class I drug according to the Biopharmaceutical Classification System (BCS) (Kimberley et al., 2002; Lindenberg et al., 2004) due to its solubility and permeability characteristics, an IVIVC can be expected for slow release formulations of this drug (Roshdy et al., 2002; Yu et al., 1996). However, we must be cautious due to the many physiological factors affecting oral absorption.

For the above reasons, a previous study (Ferrero and Jiménez-Castellanos, 2014) assessed the influence of the tablet crushing force, the pH of the dissolution medium and the agitation rate on the *in vitro* theophylline release kinetics from starch-methyl methacrylate matrix tablets. The results were compared with those of two commercial formulations of theophylline (Theo-Dur® and Theolair®). FD-HSMMA, OD-CSMMA and FD-CSMMA matrix tablets were selected on the basis of their mechanical resistance and

their similar release profiles with the marketed products. The aim of the present work was then to investigate if the matrix tablets selected from *in vitro* release studies confer adequate oral absorption and pharmacokinetic properties, as drug sustained delivery systems. Female beagle dogs were used as an animal model for evaluating theophylline absorption (Cook et al., 1990). Attention has also been focused on the possibility of predicting expected *in vivo* bioavailability characteristics from dissolution profiles (IVIVC).

2. Materials and methods

2.1. Materials

Aqueous solution (10 ml) of aminophylline (Eufilina Venosa®, BYK Elmu, Madrid, Spain) corresponding to 175.7 mg of anhydrous theophylline was used as intravenous administration. Five theophylline formulations were selected for oral administration based on the similarity of the *in vitro* release profiles (Ferrero and Jiménez-Castellanos, 2014):

- *In vitro* pH-independent release: FD-HSMMA matrix tablets (100 mg theophylline) and Theo-Dur® 100 mg (Pharmacia & Upjohn S.A., Barcelona, Spain) as reference product.
- *In vitro* pH-dependent release: OD-CSMMA and FD-CSMMA matrix tablets (175 mg theophylline) and Theolair® 175 mg (3M España S.A, Madrid, Spain) as reference product.

According to manufacturer's information and literature review (Munday and Fassihi, 1995; Shangraw, 1988), Theo-Dur® is composed of theophylline sugar pellets coated with lipid materials and cellulose acetate phthalate (CAP) and embedded into a

slowly disintegrating waxy type matrix containing additional drug. In contrast, Theolair® is formulated in the form of theophylline tablets containing lactose as soluble excipient and coated with cellulose acetate phthalate (Crombeen and De Blaey, 1983; Shangraw, 1988).

The method of preparation of the copolymers tablets is well-described in Ferrero and Jiménez-Castellanos (2014). Briefly, mixtures (500 mg) of copolymer, anhydrous theophylline (as model drug) and stearic acid (as lubricant) were directly compressed (single punch tablet machine Bonals AMT 300, Barcelona, Spain) to obtain flat-faced compacts (12 mm diameter) at a crushing force of 90-100 N.

Sodium heparin 5000 UI/ml (Rovi, Madrid, Spain), monoclonal II theophylline (Abbott, Madrid, Spain), calibrators to TDxFLx (Abbott, Madrid, Spain) at 0.0, 2.5, 5.0, 10.0, 20.0 and 40.0 μg/mL of theophylline, and controls to calibration verification of TDxFLx at 7.0, 12.0 and 26.0 μg/mL of theophylline were used as reagents.

2.2. Animals

Six healthy female beagle dogs, weighing 8.5-13 kg were housed individually in controlled conditions (temperature, humidity and light-dark cycles). The dogs did not receive food but had free access to water for 12 h before and after drug administration. The animal experimentation was approved by the Ethical Committee of Animal Experimentation of the Faculty of Veterinary Medicine from Cordoba University (Spain).

2.3. Experimental design

2.3.1. *In vivo* theophylline absorption

Each animal received the three tested sustained-release formulations (FD-HSMMA matrix tablets with 100 mg of theophylline, OD-CSMMA and FD-CSMMA matrix tablets

with 175 mg of theophylline), besides Theo-Dur®, Theolair® and an intravenous solution of theophylline (5 ml of aminophylline solution equivalent to 87.85 mg of anhydrous theophylline) in a Williams's cross-over design (Jones and Kenward, 1989). A wash-out period of two weeks was allowed between the different treatments.

Serial blood samples (5 mL) were collected from the cephalic vein at predetermined time points up to 12h for the intravenous solution and 24h for the oral formulations. All blood samples were taken in heparinized tubes (BD vacutainers® LH 143 IU, New Jersey, USA), and plasma was separated by centrifugation (JP Selecta Cemcom, Abrea, Barcelona, Spain) at 3000 rpm and immediately frozen at -20ºC (Revco, ULT 1786-3-v30, North Carolina, USA) until analysis.

2.3.2. Theophylline analytical method

Theophylline plasma concentrations were determined by a validated fluorescence polarization immunoassay analysis (FPIA) using the TDx/TDxFLx® method (Abbott™ Laboratories, Madrid, Spain) (Jolley et al., 1981). The assay was linear over plasma concentrations ranging from 0.5 to 30 μg/mL. The intraday and interday coefficients of variation ranged from 0.48% to 6.42% at the three concentrations tested (7.0, 12.0, 26.0 μg/mL). The lower limit of quantification (LLOQ) was established at 0.80 ± 0.07 μg/mL and its variation coefficient was 8.75%. The mean absolute recovery of theophylline was 100.6 ± 2.96%. The values obtained are within the limits accepted by FDA (2001) for bioanalytical methods.

2.4. Data Analysis

166 2.4.1. Pharmacokinetic analysis

167 It is known that food could induce absorption changes ("food effect") from
168 controlled-release formulations of theophylline (Cook et al., 1990; Karim, 1986; Shiu et al.,
169 1989). So, plasma levels of theophylline in fasting dogs were plotted against time, and
170 pharmacokinetic parameters were calculated by a non-compartmental method using
171 WinNonlin 5.3 (Pharsight Corporation, Mountain View, California). The area under the
172 plasma concentration *vs* time curve up to the last sampling time point, $AUC_{0\text{-}t}$, was obtained
173 using the linear and log-linear trapezoidal method. The $AUC_{0\text{-}t}$ was extrapolated to infinity
174 ($AUC_{0\text{-}\infty}$) by adding the quotient C_t/K_{el}, where C_t represents the last measured concentration
175 and K_{el} represents the apparent terminal rate constant. K_{el} was calculated by the linear
176 regression of the log-transformed concentrations of the drug in the terminal phase. The
177 half-life of the terminal elimination phase was obtained using the relationship $t_{1/2}$ =
178 $0.693/K_{el}$. The maximum value of the plasma concentration (C_{max}) and the time of
179 maximum concentration (T_{max}) were obtained directly from the data. Mean residence time
180 (*MRT*) was determined by division of *AUMC* (area under the first moment curve) by $AUC_{0\text{-}\infty}$. Absolute oral bioavailability (*F*) was calculated from plasma data using the relationship
182 $F = \left(\frac{dose_{IV} \times AUC_{0-\infty\ oral}}{dose_{oral} \times AUC_{0-\infty\ IV}}\right) \times 100.$

183 A compartmental pharmacokinetic analysis by means of the population approach
184 was also performed. Data from both the intravenous administration and the five oral
185 formulations were simultaneously modeled using NONMEM® 7.2 (Globomax, Rockville,
186 MD) (Bauer, 2011). Graphical diagnostics were assessed using Xpose version 4.2.1
187 (Jonsson and Karlsson, 1999) implemented into R version 2.14.2 and Perl speaks-
188 NONMEM (PsN) version 3.2.4 Tool-kit (Lindbom et al., 2005). The first order conditional

189 estimation method (FOCE) with interaction was used. One and two compartment models
190 with linear elimination were tested in all the cases. First-order kinetics without or with lag-
191 time were tested to describe the absorption profile. The models were parameterized in terms
192 of absorption rate constant (K_a), apparent volume of distribution (V_d), elimination clearance
193 (Cl) and bioavailability (F). Between-animal variability (BAV) evaluated for each
194 pharmacokinetic parameter was modeled exponentially, assuming a log-normal
195 distribution. Additive, proportional and combined (additive + proportional) models were
196 compared to assess the residual error (RE). To statistically distinguish between nested
197 models, the difference in the minimum value of the objective function (MOFV) was used
198 because this difference is approximately χ^2 distributed. A significance level of $p < 0.005$
199 that corresponded to a difference in MOFV of 7.879 for 1 degree of freedom was
200 considered. For non-hierarchical models, the most parsimonious model with the lowest
201 objective function according to the Akaike's Information Criterion (AIC) was chosen
202 (Yamaoka et al., 1978). Once the base model was developed, the effect of the type of
203 formulation on absorption pharmacokinetic parameters (K_a and F) was investigated with
204 NONMEM®. This covariate was tested firstly univariately on each parameter and then by
205 the forward inclusion/backward elimination procedures. Significance levels of 5%
206 (ΔMOFV=-3.841 units) and 0.1% (ΔMOFV=10.8 units) were considered during the
207 forward addition and backward elimination steps. The decrease in MOFV (-2xlog
208 likelihood), parameter precision expressed as relative standard error (RSE%), reductions in
209 BAV associated to parameters, model completion status and visual inspection of goodness-
210 of-fit plots were also considered for model selection.

211 From the final model, simulations were performed based on the final
212 pharmacokinetic estimates using 1000 individuals for each formulation to calculate the

95% prediction intervals of theophylline plasma concentrations. Whether the observations dropped into the 95% prediction interval was evaluated (visual predictive check) (Holford, 2005). Moreover, from the final pharmacokinetic parameters estimates, theophylline plasma concentrations *vs* time profiles achieved at steady-state after various dosing regimens were simulated and compared in order to establish the optimum therapeutic regimen for the formulations under study.

2.4.2. *In vitro-in vivo* correlation (IVIVC)

In vitro-in vivo correlations (IVIVC) were performed from *in vitro* dissolution and *in vivo* generated data.

The *in vitro* dissolution studies are described in detail in a previous work (Ferrero and Jiménez-Castellanos, 2014). Briefly, release experiments (6 tablets) were performed in an automatic dissolution apparatus I (Aidec, Barcelona, Spain) at a stirring rate of 100 rpm. The type of apparatus and the rotational speed were selected following the recommendations of FDA (1997) for the development of an IVIVC. To simulate the fasting *in vivo* environment, a pH change media (500 ml) was used: 0.1N HCl pH 1.2 for 1.5h; phosphate buffer (pH 2.5) for 1.5h; phosphate buffer (pH 4.5) for 1.5h; phosphate buffer (pH 7.0) for 3h and phosphate buffer (pH 7.5) for 1h, maintained at 37 ± 0.5 ºC. Ionic strength was kept constant to 0.1. Samples were extracted at regular time intervals and assayed spectrophotometrically at 272 nm.

The model-independent approach based on principles of statistical moments was used to estimate the mean dissolution time (MDT) (Podczeck, 1993). The time at which the 50% of the drug was dissolved ($t_{50\%}$) and the percentage dissolved at 4 hours (Q_{4h}) were also calculated from the drug release profiles.

The different levels of IVIVC according to the FDA recommendations (FDA, 1997) were tried, i.e.: a) level A, corresponding to the case in which the entire *in vivo* time course of the plasma drug concentration is totally predicted from the *in vitro* data; b) level B, comparing the *MDT* (*in vitro*) to the *MRT* (*in vivo*); c) level C, establishing a single point relationship between a dissolution parameter, either $t_{50\%}$ or Q_{4h} (*in vitro*) and a pharmacokinetic parameter, e.g., *AUC*, C_{max} or T_{max} (*in vivo*). In order to investigate the IVIVC of level A, a deconvolution analysis by means of the Wagner-Nelson method (Wagner and Nelson, 1964) was also applied to characterize the *in vivo* drug absorption profile and to calculate the absorbed percentages of theophylline from each oral formulation. Mean fractions dissolved and mean fractions absorbed were used to investigate the level A IVIVC.

2.4.3. Statistical analysis

Statistical comparisons between formulations of geometric means of the estimated parameters, by the individual approach, were performed by means of a two-way analysis of variance (ANOVA), taking into account the formulation and the animal as fixed and random factors, respectively. It should be noted that statistical analysis was performed using all data together. The dose normalized values were compared in the case of C_{max} and *AUC*. The SPSS® 17.0 software was used (SPSS Inc., Chicago IL).

3. Results and discussion

3.1. *In vivo* bioavailability studies

The mean plasma concentration-time profiles (log-scale) of theophylline after intravenous and oral administration of the formulations are displayed in Fig. 1. In order to determine the absolute bioavailability of the sustained-release theophylline formulations, a theophylline solution was given intravenously. The monoexponential decay found in our study after intravenous administration (Fig. 1A) is in accordance with previous studies in dogs (Liaw et al., 1990; Shiu et al., 1989; Tse and Szeto, 1982). Nevertheless, a biexponential decay has also been reported (Alberola et al., 1993; Bach et al., 2004; Kuze et al., 1988; Mitenko and Ogilvie, 1972; Yu et al., 1996). This discrepancy could possibly be due to differences in the sampling scheme at the early post-dosing sampling times that, otherwise, is crucial to accurately describe the initial fast theophylline distribution to peripheral tissues.

The oral administration of the three tested formulations to beagle dogs resulted in very similar patterns in comparison with the respective reference products (Fig. 1B, 1C) in terms of duration of plasma levels over the limit of quantification. All theophylline concentrations were quantifiable from the first sampling time at 0.5 to 12 h. However, some dogs (1 for Theo-Dur®, 2 for FD-HSMMA, 3 for OD-CSMMA and Theolair® and 4 for FD-CSMMA) showed quantifiable plasma levels during more time. These differences between animals explain the higher variability observed for FD-HSMMA formulation at 24h and indicate that drug elimination can vary markedly among subjects, as occurs in humans (Conard et al., 1982). Otherwise, non signs of toxicity associated to theophylline administration were observed during the study. In any case, after oral administration, theophylline concentrations increased up to a peak value and then, a monoexponential decline was observed for all the formulations. As it should be expected, visual inspection of plots of Fig. 1B, 1C suggested similar absorption/disposition profiles for FD-HSMMA *vs*

Theo-Dur® and for OD-CSMMA and FD-CSMMA *vs* Theolair®, consistent with the behaviour observed in the *in vitro* release studies (Ferrero and Jiménez-Castellanos, 2014).

The main pharmacokinetic parameters estimated for the different formulations by the non-compartmental approach are summarized in Tables 1 and 2. No statistically significant differences were found for any of the pharmacokinetic parameters when all formulations were compared ($p>0.05$). However, the median T_{max} values range from 3.0 to 5.5 h, being the increasing rank order: FD-HSMMA < Theo-Dur® ≈ OD-CSMMA < Theolair® < FD-CSMMA. These values are consistent with those previously reported in the literature (Conard et al., 1982; Mengozzi et al., 1998; Ochoa et al., 2010). Moreover, the tested matrix tablets prolong the release/absorption of theophylline if we compare our results with the data obtained by El-Sayed et al. (1996) and Qiu et al. (1998) for a theophylline solution (T_{max} 1 and 1.06 h, respectively), Tse and Szeto (1982) for Elixofilina (T_{max} 1.5 h) and Turkoglu et al. (1994) for uncoated pellets (T_{max} 1.7 h). For matrix tablets, Hayashi et al. (2007) obtained a T_{max} value of 4 h, whereas Ochoa et al. (2010) reported values ranging from 3.17 to 6 h depending on the binder used. In both cases a melt granulation technique was used to obtain granules with 200 mg of theophylline.

The trend observed in C_{max}/AUC values is in agreement with T_{max}, being the ranking order of absorption rates from fastest to slowest: FD-HSMMA > Theo-Dur® ≈ OD-CSMMA > FD-CSMMA ≥ Theolair®. MRT values are higher than the intravenous data (7.49 ± 3.48 h) indicating that formulations prolong the plasma concentrations of theophylline.

Due to the less robust estimation of the apparent half-life values, highly dependent on the quantifiable concentrations at the monoexponential terminal phase, these values are not taken into account for discussion in the current work. As expected, the C_{max} and AUC

values found for Theo-Dur® and FD-HSMMA formulations (100 mg) are lower than those for Theolair® and CSMMA formulations (175 mg). However, no statistically significant differences are observed when C_{max} and AUC parameters are normalized by dose ($p>0.05$).

The absolute bioavailability (F) values show a good absorption of the drug, proving that more than 72% of theophylline is released/absorbed from the tablet. Even the tested formulations FD-HSMMA and OD-CSMMA show slightly higher F than their respective reference products.

In order to clarify some of the individual differences found with the non-compartmental analysis and to extend the results provided by this method, a compartmental analysis through the population approach was performed. The population approach allows a simultaneous analysis of data of all formulations so that additional information *vs* the classical individual approach (where data of each formulation must be analysed independently) can be provided. The compartmental analysis indicates a one-compartment model with first order absorption and elimination processes without lag-time as the best to describe the absorption/disposition of theophylline from intravenous and oral formulations. Between-animal variability (BAV) could be associated to plasma clearance (Cl) and absorption rate constant (K_a). Residual error (RE) was best described by an additive-proportional error model. The univariate inclusion of four different absorption rate constants for FD-HSMMA/Theo-Dur® and OD-CSMMA, FD-CSMMA/Theolair®, respectively, provided a statistically significant decrease of the minimum objective function value (MOFV) ($p<0.005$). After the addition of a different K_a value for Theolair®, OD-CSMMA and FD-CSMMA, respectively, the corresponding reductions in BAV associated to K_a were of 4.6% (Theolair®), 16.18% (OD-CSMMA) and 24.75% (FD-CSMMA). The

inclusion of three different bioavailability (F) values for FD-HSMMA/Theo-Dur®, OD-CSMMA and FD-CSMMA/Theolair®, respectively, also improved the model fit ($p<0.005$). The backward elimination of each one of these covariates increased significantly the MOFV ($p<0.001$). The final absorption/disposition parameters are reported in Table 3. The disposition parameters (Cl and V_d) are in agreement with those previously reported (Yu et al., 1996; Mengozzi et al., 1998). The internal validation through a visual predictive check from the final pharmacokinetic parameter estimates confirms the final model to have good predictive properties of the original data (Fig. 2-3). Figures 2-3 confirm that most of the observed data fall into the 95% prediction interval, less than 5% of the observed data being above or below it. Therefore, the population compartmental approach allows to find statistical significant differences ($p<0.05$) between the absorption rate constants, being the ranking order from fastest to slowest: FD-HSMMA ≈ Theo-Dur® > OD-CSMMA > Theolair® > FD-CSMMA. Moreover, the compartmental analysis allows statistically significant different ($p<0.05$) bioavailability values in the order Theo-Dur® ≈ FD-HSMMA < FD-CSMMA ≈ Theolair® < OD-CSMMA.

Fig. 4 shows simulated theophylline plasma concentration *vs* time profiles after repeated administrations of the three tested formulations and the corresponding reference products, superimposed with the therapeutic range values for theophylline. The concentration *vs* time profiles simulated after repeated oral dosing show that the freeze-dried formulations compare well with their respective reference products. So, a three times daily regimen for FD-HSMMA and a twice daily regimen for FD-CSMMA could be proposed to maintain theophylline plasma concentrations in the dog within the therapeutic range (5-15 μg/mL). As expected, because of the higher absorption rate, in the case of OD-

CSMMA matrices, a two times daily regimen would provide more fluctuating theophylline concentrations with mean peak concentrations at steady-state higher than 15 but lower than 20 μg/mL, although toxicity signs seem to appear over 20 μg/mL. Hence, we can conclude that, even with a twice daily dosage regimen, FD-CSMMA matrices have acceptable sustained release characteristics, similar to the commercial Theolair® tablets (Conard et al., 1982).

Although the trends in the rate and/or extent of theophylline absorption from the formulations in dogs are expected to be maintained in humans (Cook et al., 1990), an interspecies scaling approach (Gascón et al., 1994) would be needed to predict the best dose regimen in humans from the results obtained in this preclinical research.

3.2. *In vitro-in vivo* correlations

The *in vitro-in vivo* correlations for the different formulations were explored by comparing *in vivo* drug release obtained from deconvolution with the *in vitro* release data.

The *in vitro* dissolution parameters estimated from the different formulations (Table 4) are compared according to their pH-independent (FD-HSMMA *vs* Theo-Dur®) or pH-dependent (OD-CSMMA and FD-CSMMA *vs* Theolair®) character. Theo-Dur® show lower percentages dissolved at 4 hours (Q_{4h}) values and higher mean dissolution times (MDT) and $t_{50\%}$ values than FD-HSMMA formulation. These results are in agreement with the faster drug release rates reported for FD-HSMMA matrices compared with TheoDur® (Ferrero and Jiménez-Castellanos, 2014). This behaviour was attributed to the different formulation of these systems, as the major part of theophylline in TheoDur® is contained in pellets embedded in the matrix. Although the tendency in T_{max}, AUC and F values (Table 1) would indicate also a faster *in vivo* release/absorption for FD-HSMMA tablets, it could not

be confirmed by the population approach as similar K_a values were found for both formulations (Table 3).

Concerning pH-dependent formulations, the *in vitro* results (Table 4) show also lower Q_{4h} values and higher MDT and $t_{50\%}$ values for Theolair® compared with OD-CSMMA formulation. In contrast, FD-CSMMA formulation shows closer values to Theolair®, in agreement with the tendency reported for the drug release rates (Ferrero and Jiménez-Castellanos, 2014). These *in vitro* results are consistent with the trend observed in the *in vivo* parameters T_{max}, AUC and F (Table 2) and with the highest absorption (K_a and F values) described for OD-CSMMA by the compartmental population approach (Table 3).

For the tested matrices, the order in the absorption rate FD-HSMMA > OD-CSMMA > FD-CSMMA is consistent with the tendency reported for the drug release rates (Ferrero and Jiménez-Castellanos, 2014) and could be explained by formulation factors such as polymer nature and tablet porous network. The strongly retarded drug release/absorption of CSMMA and FD matrices could be attributed to the better binding properties of these derivatives. Moreover, OD matrices were characterized by less tortuous pore networks than their homologous freeze-dried, which explains the faster drug release/absorption from those matrices. Hence, the absorption kinetics seems to be determined by the same variables affecting the drug release kinetics.

As mentioned in the introduction, theophylline belongs to Class I of BCS, being a good candidate to develop a level A IVIVC when more than two extended release formulations are involved. Such type of IVIVC is generally linear (FDA, 1997) and has been previously reported for theophylline extended release formulations (Ochoa et al., 2010). However, in the present study, the percentage of drug absorbed *in vivo* from TheoDur® and Theolair® can not be predicted point-to-point from the percentage of *in vitro*

drug released. A more acceptable linear fitting (r^2 = 0.93-0.95) is obtained in the case of the three tested matrices (Figure 5). This could be due to: a) the different formulation and method of manufacture of the standard and test systems (Ferrero and Jiménez-Castellanos, 2014; Kortejarvi et al., 2002; Nabais et al., 2007); b) the pH-dependent or -independent character of the formulations (Cutler et al., 1997; Ferrero and Jiménez-Castellanos, 2014; Ochoa et al., 2010). The certain degree of curvature exhibited by the profiles is likely a direct result of the difference in release kinetics between *in vitro* and *in vivo*.

Although we also failed to obtain an acceptable level B correlation for all formulations, a significant level C IVIV correlation (r^2 = 0.9411, $p < 0.05$) can be found between $t_{50\%}$ (*in vitro*) and AUC/D (*in vivo*) (Figure 6A). The higher deviation of linearity of Theolair® could be a consequence of its pH-dependent release profile (Ferrero and Jiménez-Castellanos, 2014)**.** The fit is better (r^2 = 0.9958, $p < 0.05$) when only the tested matrices are compared (Figure 6B), confirming the importance of the influence of the formulation design. As expected, an increase in the *in vitro* variable (time for 50% release) is associated with a decrease in the *in vivo* variable (AUC/D). Due to the therapeutic relevance of this *in vivo* parameter (directly related to the extent of absorption), this correlation may have and advantage over level B measures (Cutler et al., 1997).

Finally, it is interesting to note that *in vivo* drug absorption rates from all formulations were faster than the *in vitro* drug release rates, implying that the gastrointestinal physiological conditions, which are more extreme than those in the *in vitro* dissolution tests, enhanced release and, in turn, the absorption process. So, a most bio-relevant media (with bile salts, enzymes, etc.) would be required in order to totally predict the *in vivo* release/absorption profile from the *in vitro* release data.

CONCLUSIONS

In conclusion, potato starch-methyl methacrylate polymers are interesting excipients for sustained drug release in solid oral dosage forms. In addition to the easy manufacture of tablets by direct compression, the results show extended drug release/absorption of the tested formulations *in vivo* by comparing their pharmacokinetic parameters with the commercially available Theo-Dur® and Theolair® in beagle dogs under fasting conditions. Hence, these materials could be a good alternative to incorporate drug candidates of similar physico-chemical properties to theophylline to maintain therapeutic plasma concentrations during more lasting periods of time without signs of toxicity. FD-CSMMA was the derivative that provided a better control of drug release/absorption process and a twice daily dosage regimen would be recommended on the basis of the simulation studies in dogs.

Moreover, the simpler formulation of starch-methyl methacrylate matrices compared with the marketed products allowed establishing stronger relationships between *in vitro* and *in vivo* data. The quantitative correlation between $t_{50\%}$ *in vitro* and AUC/D *in vivo* could be regarded as a first step to predict the extent of absorption from dissolution data. Nevertheless, further investigation will be required on the most bio-relevant media in order to totally predict the *in vivo* release/absorption profile from the *in vitro* release data.

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Table 1. Pharmacokinetic parameters of theophylline (mean *(SD)*, n=6) in beagle dogs after single oral administration of FD-HSMMA (100 mg) and Theo-Dur® (100 mg) formulations.

Parameter *(Units)*	**FD-HSMMA**	**Theo-Dur®**
C_{max} *(µg/mL)*	8.10 *(2.21)*	8.16 *(2.07)*
C_{max}/D *(L^{-1})*	0.0810 *(0.0221)*	0.0816 *(0.0207)*
AUC *(µg·h/mL)*	90.60 *(54.17)*	78.96 *(20.50)*
AUC/D *(h/L)*	0.9060 *(0.5417)*	0.7896 *(0.2050)*
F	0.771 *(0.264)*	0.718 *(0.123)*
$t_{1/2}$ *(h)*	6.38 *(3.88)*	5.28 *(1.17)*
T_{max}* *(h)*	3.00 *(2.02-4.97)*	3.51 *(2.00-5.02)*
C_{max}/AUC *(h^{-1})*	0.1055 *(0.0347)*	0.1040 *(0.0121)*
MRT *(h)*	10.23 *(5.14)*	9.53 *(1.40)*

C_{max} = peak plasma concentration; C_{max}/D = normalised by dose peak plasma concentration; AUC = area under the plasma-concentration *vs* time curve; AUC/D = normalised by dose area under the plasma-concentration *vs* time curve; F = bioavailability; $t_{1/2}$ = apparent half-life; T_{max}= time to peak plasma concentration; MRT = mean residence time.

*Median (minimum-maximum) values are given for T_{max}.

Table 2. Pharmacokinetic parameters of theophylline (mean *(SD)*, n=6) in beagle dogs after single oral administration of OD-CSMMA (175 mg), FD-CSMMA (175 mg) and Theolair® (175 mg) formulations.

Parameter *(Units)*	**OD-CSMMA**	**FD-CSMMA**	**Theolair®**
C_{max} *(µg/mL)*	16.86 *(1.69)*	12.40 *(3.40)*	13.18 *(2.58)*
C_{max}/D *(L^{-1})*	0.0963 *(0.0097)*	0.0708 *(0.0194)*	0.0753 *(0.0147)*
AUC *(µg·h/mL)*	168.97 *(41.25)*	148.32 *(45.69)*	160.30 *(23.51)*
AUC/D *(h/L)*	0.9655 *(0.2357)*	0.8476 *(0.2611)*	0.9160 *(0.1344)*
F	0.896 *(0.253)*	0.756 *(0.151)*	0.856 *(0.179)*
$t_{1/2}$ *(h)*	4.75 *(1.69)*	5.57 *(1.15)*	6.55 *(1.69)*
T_{max}* *(h)*	3.53 *(1.65-6.00)*	5.54 *(2.00-8.00)*	4.00 *(2.13-8.07)*
C_{max}/AUC *(h^{-1})*	0.1037 *(0.0212)*	0.0863 *(0.0146)*	0.0849 *(0.0264)*
MRT *(h)*	8.77 *(2.15)*	10.78 *(1.90)*	11.52 *(2.61)*

C_{max} = peak plasma concentration; C_{max}/D = normalised by dose peak plasma concentration; AUC = area under the plasma-concentration *vs* time curve; AUC/D = normalised by dose area under the plasma-concentration *vs* time curve; F = bioavailability; $t_{1/2}$ = apparent half-life; T_{max}= time to peak plasma concentration; MRT = mean residence time.

*Median (minimum-maximum) values are given for T_{max}.

Table 3. Mean *(RSE%)* values of theophylline pharmacokinetic parameters estimated by the population approach.

Parameter *(Units)*	**Value *(RSE%)***
Disposition parameters	
Cl *(L/h)*	1.01 *(8.67)*
V_d *(L)*	6.47 *(4.87)*
Absorption parameters	
$K_{aTheo\text{-}Dur®}$ *(h^{-1})*	0.452 *(17.63)*
$K_{aFD\text{-}HSMMA}$ *(h^{-1})*	0.452 *(17.63)*
$K_{aOD\text{-}CSMMA}$ *(h^{-1})*	0.417 *(15.97)*
$K_{aFD\text{-}CSMMA}$ *(h^{-1})*	0.238 *(20.88)*
$K_{aTheolair®}$ *(h^{-1})*	0.292 *(24.42)*
$F_{Theo\text{-}Dur®}$	0.788 *(6.66)*
$F_{FD\text{-}HSMMA}$	0.788 *(6.66)*
$F_{OD\text{-}CSMMA}$	0.963 *(5.14)*
$F_{FD\text{-}CSMMA}$	0.864 *(3.65)*
$F_{Theolair®}$	0.864 *(3.65)*
Between-animal variability	
BAV_{Cl} (%)	24.74 *(36.93)*
BAV_{Ka} (%)	17.29 *(46.82)*
Residual error	
Additive (µg/ml)	1.18 *(24.66)*
Proportional (%)	18.0 *(28.67)*

Cl: total plasma clearance; V_d: central compartment distribution volume; K_a: absorption rate constant; F: absolute bioavailability; BAV: between-animal variability, expressed as coefficient of variation; Residual error, expressed as standard deviation (additive) and coefficient of variation (proportional). Relative standard errors of all parameters are given in parenthesis *(RSE%)*.

Table 4. *In vitro* dissolution parameter estimates of the three tested (FD-HSMMA -100 mg-, OD-CSMMA -175 mg- and FD-CSMMA -175 mg-) and the two reference theophylline (Theo-Dur® -100 mg- and Theolair® -175 mg-) formulations.

Parameter* *(Units)*	FD-HSMMA	Theo-Dur®	OD-CSMMA	FD-CSMMA	Theolair®
MDT *(h)*	2.54 *(0.21)*	3.13 *(0.17)*	2.83 *(0.92)*	3.31 *(0.33)*	4.04 *(0.90)*
$t_{50\%}$ *(h)*	4.1 *(0.45)*	7.3 *(1.02)*	2.8 *(0.17)*	5.7 *(0.63)*	4.9 *(0.19)*
Q_{4h} *(%)*	49.92 *(3.25)*	34.53 *(2.85)*	57.93 *(4.67)*	38.20 *(1.57)*	36.24 *(2.27)*

MDT = mean dissolution time; $t_{50\%}$ = time at which the 50% of the drug was dissolved; Q_{4h} = percentage dissolved at 4 hours.

*Mean values of six replicates

Figure captions

Fig. 1. Mean ± SD (n=6) plasma concentration-time profiles of theophylline in beagle dogs after intravenous administration of theophylline solution (A) and oral administration of FD-HSMMA and Theo-Dur® (100 mg) (B) and OD-CSMMA, FD-CSMMA and Theolair® (175 mg) (C).

Fig. 2. Superimposed values of the observed (triangles) and simulated plasma concentrations (μg/mL) *vs* time profiles after intravenous administration of theophylline. Mean and 95% confidence intervals obtained from 1000 simulations of theophylline plasma concentration-time profiles. Solid line (mean predictions, 50^{th} percentile). Dashed lines (2.5^{th} and 97.5^{th} percentiles); (Visual predictive check, VPC).

Fig. 3. Superimposed values of the observed (triangles) and simulated theophylline plasma concentrations (μg/mL) *vs* time profiles after oral administration of FD-CSMMA, OD-CSMMA, Theolair® and FD-HSMMA, Theo-Dur® formulations. Mean and 95% confidence intervals obtained from 1000 simulations of theophylline plasma concentration-time profiles. Solid line (mean predictions, 50^{th} percentile). Dashed lines (2.5^{th} and 97.5^{th} percentiles); (Visual predictive check, VPC).

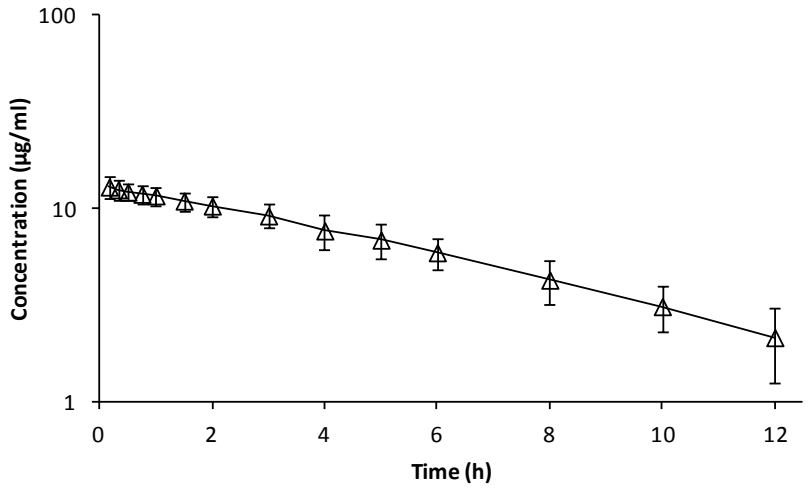
Fig. 4. Steady-state simulated theophylline plasma concentrations after FD-HSMMA (100 mg), Theo-Dur® (100 mg), OD-CSMMA (175 mg), FD-CSMMA (175 mg) and Theolair® (175 mg) repeated oral administrations to beagle dogs.

Fig. 5. Linear correlation plots for percentage of *in vivo* dose absorbed and percentage of *in vitro* dose released from FD-HSMMA (A), OD-CSMMA (B) and FD-CSMMA (C) matrices at the same time (up to the maximum amount of drug absorbed).

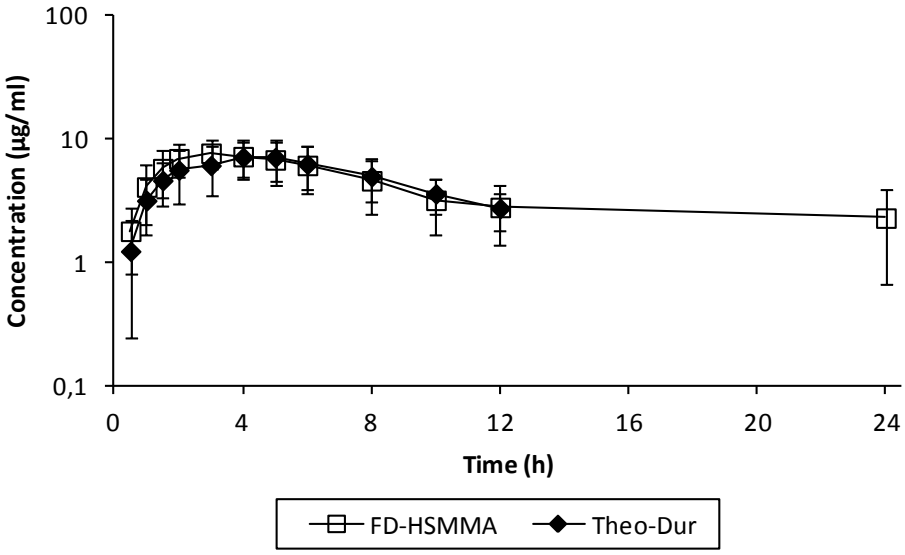
Fig. 6. Quantitative correlation between $t_{50\%}$ and AUC/D for all formulations (A) and tested formulations (B). The lines represent the best correlation, based on linear regression analysis.

Figure 1

A)


100
10
1
Concentration (µg/ml)
0
2
4
6
8
10
12
Time (h)

B)


100
10
1
0,1
Concentration (µg/ml)
0
4
8
12
16
20
24
Time (h)
FD-HSMMA
Theo-Dur

C)

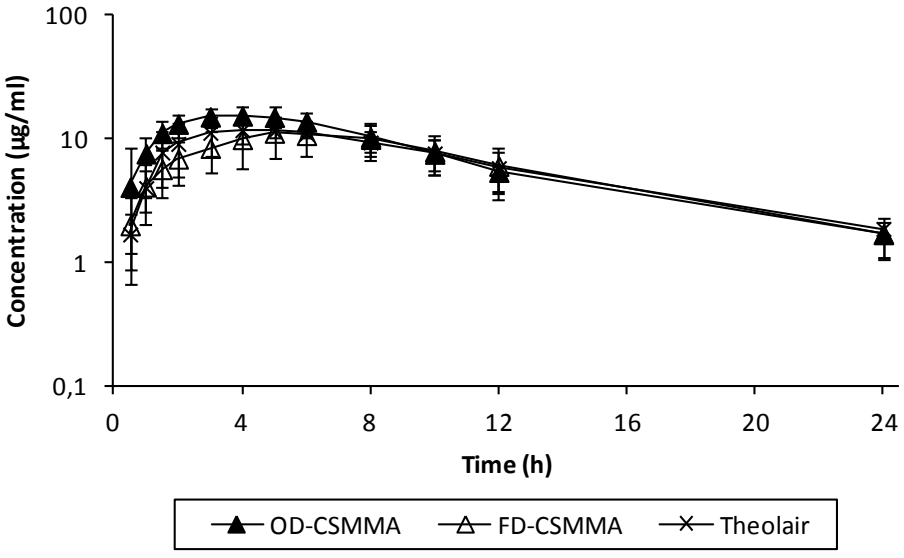

100
10
1
0,1
Concentration (µg/ml)
0
4
8
12
16
20
24
Time (h)
OD-CSMMA
FD-CSMMA
Theolair

Figure 2

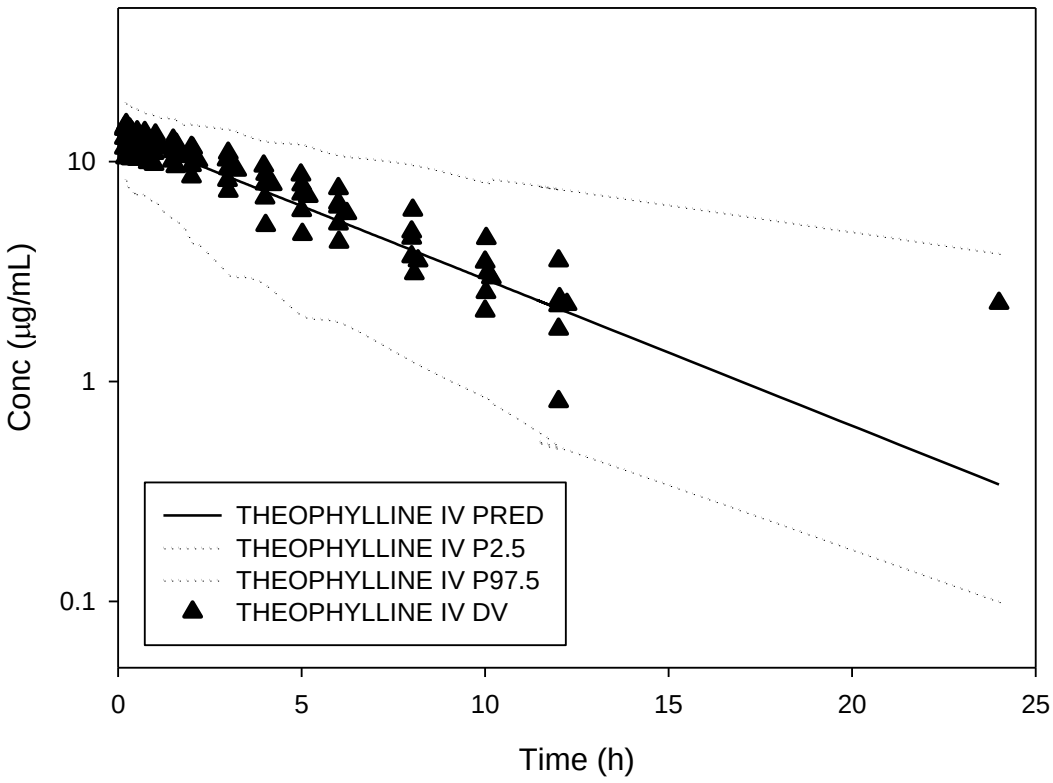

Conc (µg/mL)
10
1
0.1
0
5
10
15
20
25
Time (h)
THEOPHYLLINE IV PRED
THEOPHYLLINE IV P2.5
THEOPHYLLINE IV P97.5
THEOPHYLLINE IV DV

Figure 3

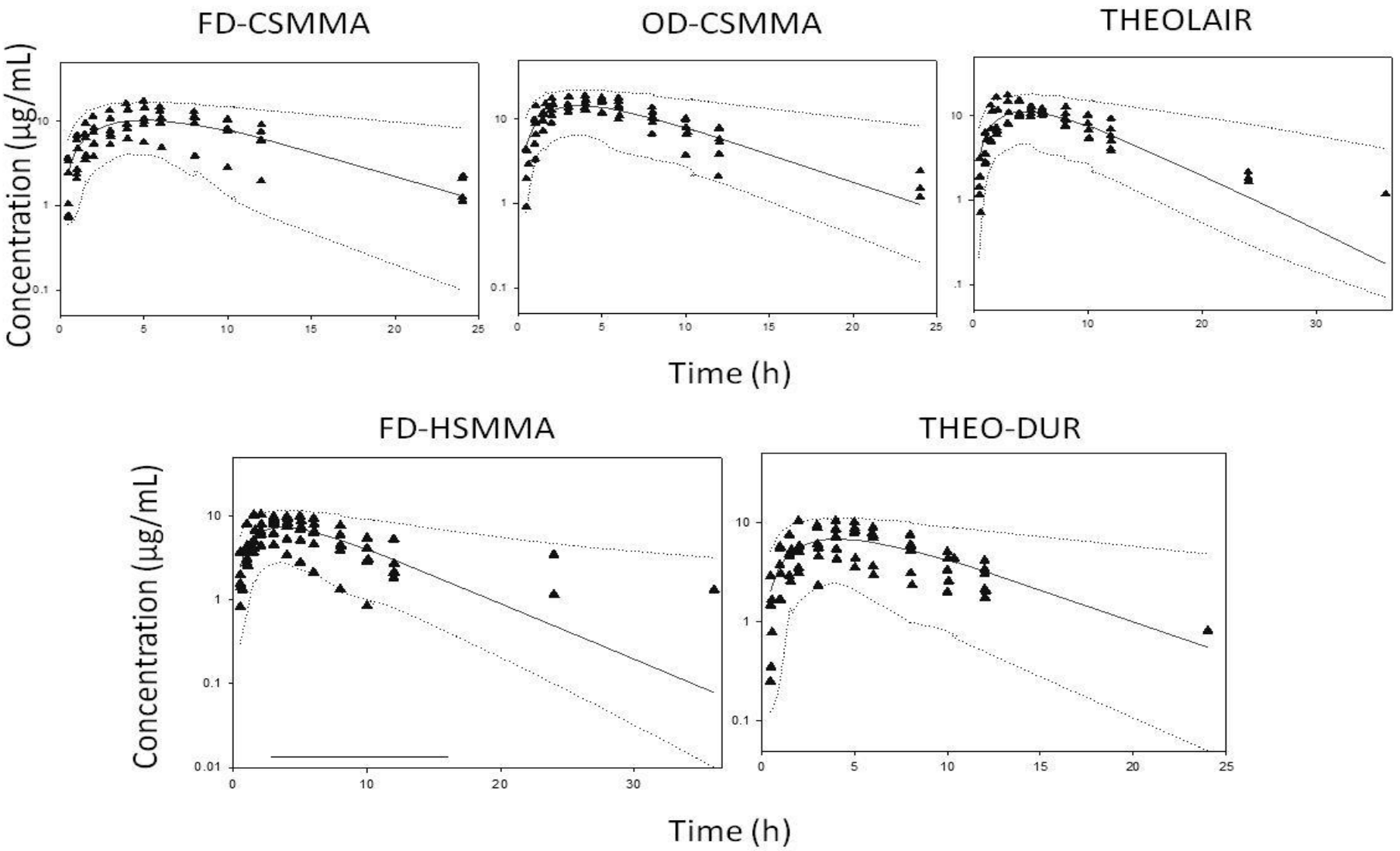
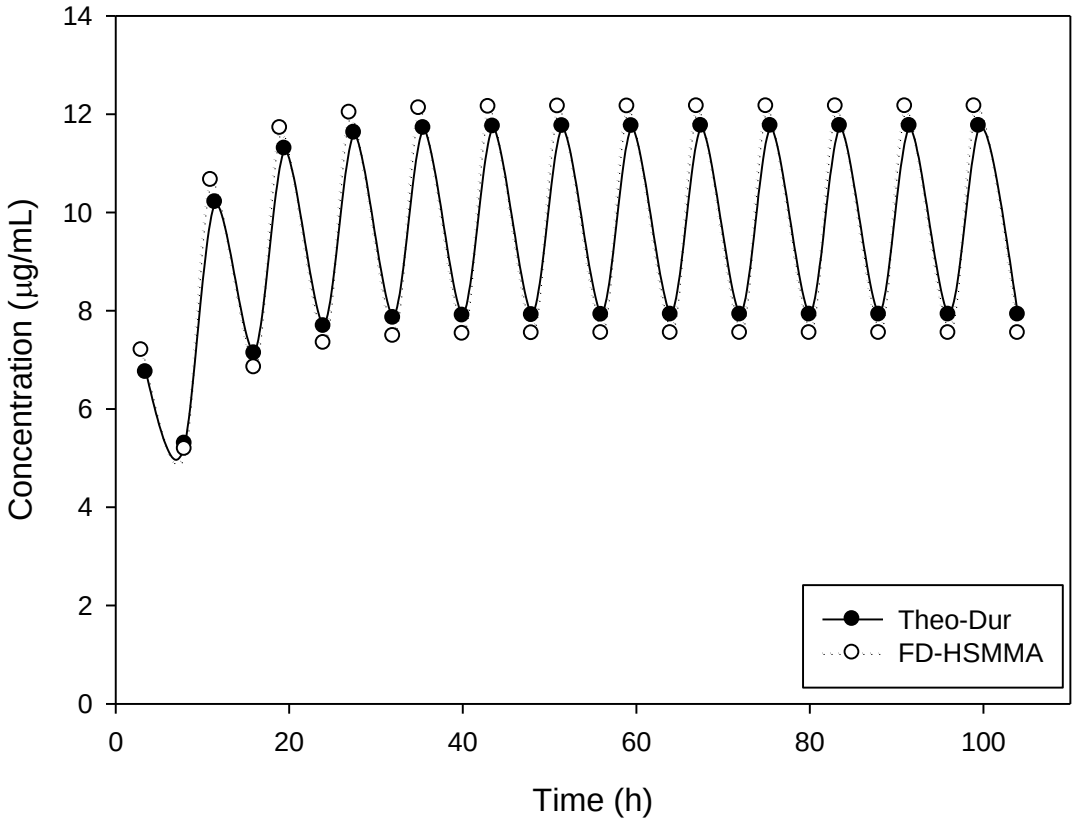

FD-CSMMA
OD-CSMMA
THEOLAIR
Concentration (µg/mL)
Time (h)
FD-HSMMA
THEO-DUR
Concentration (µg/mL)
Time (h)

Figure 4


Concentration (μg/mL)
Time (h)
Theo-Dur
FD-HSMMA

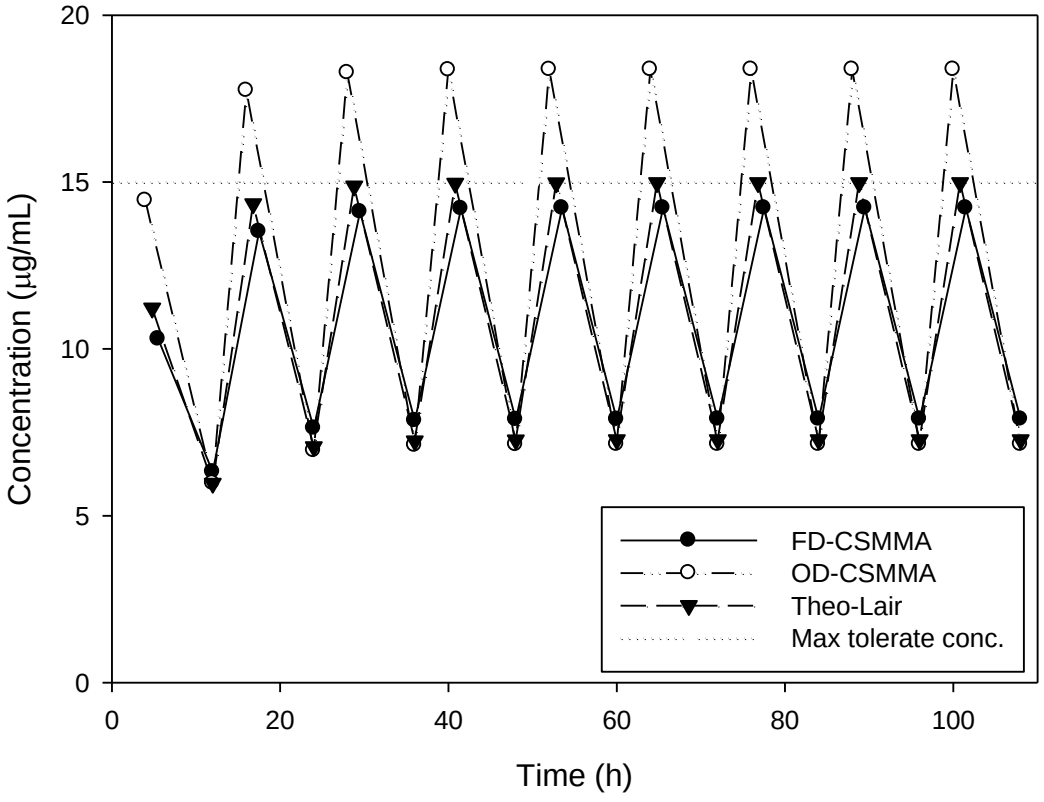
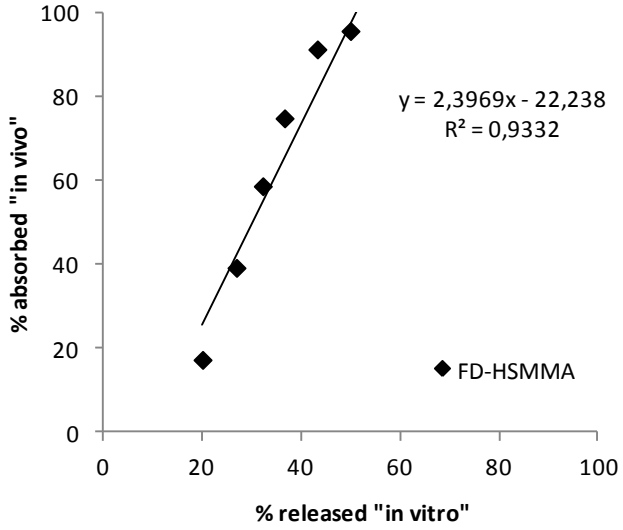
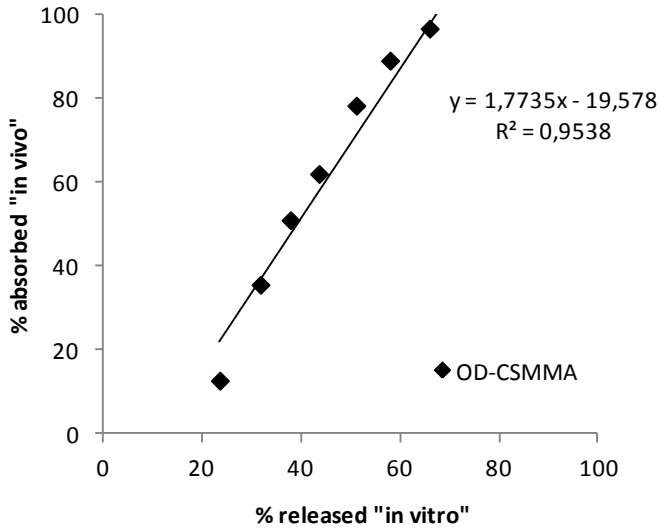

Concentration (μg/mL)
Time (h)
FD-CSMMA
OD-CSMMA
Theo-Lair
Max tolerate conc.

Figure 5

A)


y = 2,3969x - 22,238
R² = 0,9332
FD-HSMMA
% absorbed "in vivo"
% released "in vitro"
0
20
40
60
80
100

B)


y = 1,7735x - 19,578
R² = 0,9538
OD-CSMMA
% absorbed "in vivo"
% released "in vitro"
0
20
40
60
80
100

C)

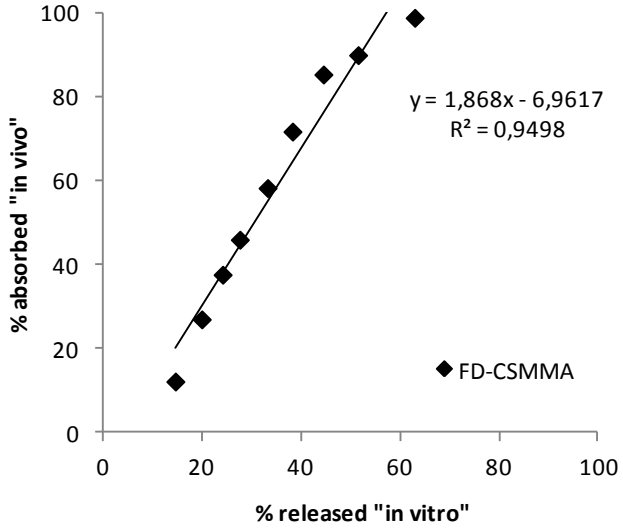
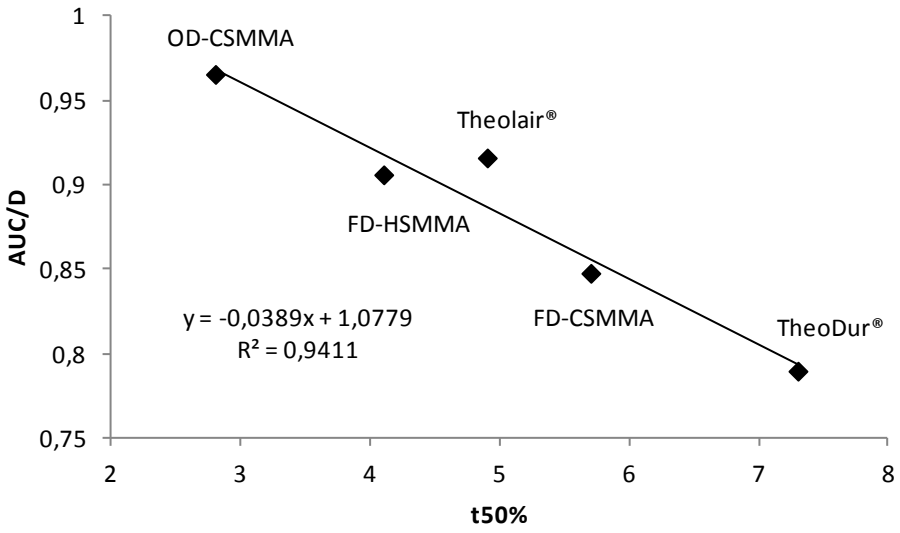
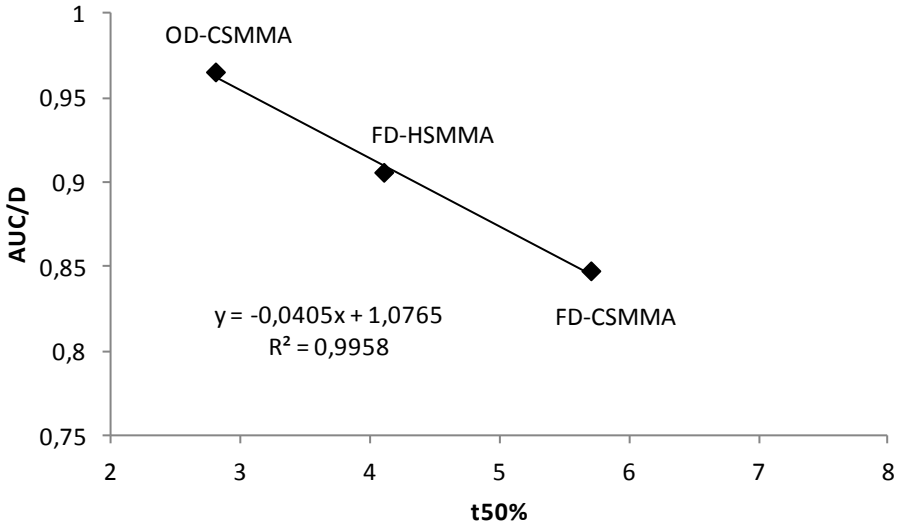

y = 1,868x - 6,9617
R² = 0,9498
FD-CSMMA
% absorbed "in vivo"
% released "in vitro"
0
20
40
60
80
100

Figure 6

A)


1
0,95
0,9
0,85
0,8
0,75
AUC/D
OD-CSMMA
Theolair®
FD-HSMMA
FD-CSMMA
TheoDur®
y = -0,0389x + 1,0779
R² = 0,9411
2
3
4
5
6
7
8
t50%

B)


1
0,95
0,9
0,85
0,8
0,75
AUC/D
OD-CSMMA
FD-HSMMA
FD-CSMMA
y = -0,0405x + 1,0765
R² = 0,9958
2
3
4
5
6
7
8
t50%