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Development of intestinal colonic drug delivery systems for diverticular disease: A QbD approach

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ABSTRACT

This study aimed to advance the development of intestinal colon-coated sustained-release matrix tablets of metronidazole for diverticulitis treatment, employing the Quality by Design (QbD) methodology. Comprehensive Risk analysis and Risk evaluation were conducted to assess the potential risks associated with Critical Material Attributes (CMA) and Critical Process Parameters (CPP). Ishikawa diagram, color-coded risk classification and the Risk Priority Number (RPN) were used as tools for risk evaluation. A Design of Experiments (DoE) was executed using a fractional factorial design, incorporating five key factors derived from the Risk analysis and Risk evaluation. Two levels and a central point were established for each factor, resulting in 28 batches of coated tablets. The manufacturing process involved direct compression, followed by a coating process using pH-dependent or time-dependent polymers. Characterization and dissolution studies were conducted on all batches, and the obtained results underwent analysis of variance (ANOVA).

The findings demonstrated the robustness and reproducibility of both the direct compression and coating processes. Statistical analysis identified HPMC/chitosan ratio, blending time, coating polymer, and coating weight gain as factors significantly impacting drug release. A Design Space was established to delineate the interplay of these factors, offering insights into various combinations influencing drug release behavior. Thus, the design space for 10 % weight gain formulations includes a range of HPMC/CH ratios between 2.7–3 and mixing times between 10 and 12 min; for 20 % weight gain formulations it includes a range of HPMC/CH ratios up to 2 and mixing times between 10 and 16 min. Multiple Linear Regression between technological and biopharmaceutical variables were optimized facilitating scale-up operations. Batches with a 10 % weight increase and varied HPMC viscosity grades and coating polymers achieve ~50 % drug release at 24 h; however, batches with a 20 % weight increase along, with either high proportions of HPMC and short blending times or low proportions of HPMC and longer blending times, achieve slow release of metronidazole. This study contributes to optimizing metronidazole colonic delivery systems, enhancing their potential efficacy in diverticulitis treatment.

1. Introduction

Colon diseases, such as diverticulitis, often caused by microbial infections, can be more effectively treated by delivering antibiotics directly to the infected site. Localized release prevents the absorption of antibiotics into the bloodstream and reduces the risk of potential side effects associated with systemic treatment. Moreover, slow and continued release of antibiotics at the infection site is beneficial in the treatment of diverticulitis, a gastrointestinal problem that is accompanied by continued constipation and that sometimes results in an infection (Singh et al., 2016).

Metronidazole is a synthetic nitroimidazole with activity against anaerobic bacteria (Bendesky and Menéndez, 2009). It is a preferred antibiotic for the non-invasive treatment of diverticulitis, typically administered at 500 mg every 8 h orally (Biondo et al., 2014; González Plo et al., 2020). Near-complete absorption of metronidazole along the upper gastrointestinal tract after its oral administration hinders it from reaching the colon (Lau et al., 1992; Lamp et al., 1999). Maximizing metronidazole release in the colon is crucial for effective treatment of diverticulitis, preventing systemic absorption and potential associated side effects. The development of colon drug delivery systems research has explored innovative materials and technologies (Sinha et al., 2005;

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Table 1
Levels given to each factor introduced and studied in the DoE.

Factor	Condition	Level
HPMC grade	HPMC K15	−1
	HPMC K35	+1
Ratio HPMC/CH	Higher HPMC proportion	3.00 (3:1)
	Higher CH proportion	0.33 (1:3)
Blending time (min)	Longer blending times	20
	Shorter blending times	10
Coating agent	Eudragit® FS 30D	−1
	Eudragit® RL 30D	+1
%ΔW	Thicker coats	20
	Thinner coats	10

CH: Chitosan; %ΔW: percentage of increase in total weight after coating.

Table 2
Design matrix for fractional factorial design used in the DoE.

	Factors resulting from the FMEA analysis					
BATCHES*	HPMC grade	% HPMC	% CH	Blending time (min)	Coating agent	%Δ W
F1, F18, F22	K15	8	24	20	RL30D	10
F8, F9, F15	K35	24	8	10	RL30D	10
F6, F7, F12	K15	8	24	10	RL30D	20
F2, F20, F25	K35	24	8	20	RL30D	20
F21	K15	16	16	15	RL30D	15
F14	K35	16	16	15	RL30D	15
F4, F5, F11	K15	24	8	20	FS30D	10
F13, F16, F17	K35	8	24	10	FS30D	10
F3, F23, F27	K15	24	8	10	FS30D	20
F19, F26, F28	K35	8	24	20	FS30D	20
F24	K15	16	16	15	FS30D	15
F10	K35	16	16	15	FS30D	15

* Each row corresponds with the three replicated outputs from the experimental design. Raws with a single batch correspond with the central points. The line separates time-dependent and pH-dependent formulations. %HPMC: percentage of HPMC per tablet; %CH: percentage of chitosan per tablet; %ΔW: percentage of increase in total weight after coating.

Umar et al., 2008; Li et al., 2015; Arévalo-Pérez et al., 2020). Strategies that use only one stimulus for drug release tend to have greater variability. Therefore, it is common to combine them so that release is stimulated either sequentially (where release occurs in a specific order) or in parallel (where multiple release mechanisms act simultaneously). Currently, promising lines of research include colon-targeted oral multi-stimulus nanosystems, although they have yet to show significant results in human studies (Chaubey et al., 2020; Brar et al., 2021; Hoang et al., 2021; Hou et al., 2022; McCoubrey et al., 2023). Multi-stimulus strategies in tablets, capsules, and granules have been most successful in delivering drugs to the colon. (Varum et al., 2020a, b; McCoubrey et al., 2023). Matrix tablets composed of hydrophilic polymers and coated with functional polymers may be a candidate approach achieving colonic drug release (Behera, 2024). Matrices form an entangled structure (gel) when they come in contact with an aqueous medium, modulating the release rates of the tablet drug content (Helin-Tanninen and Fenton-May, 2015).

Hydroxypropyl methylcellulose (HPMC) and chitosan (CH), both hydrophilic polymers forming thick hydrogels, are commonly employed in matrix tablets development for their sustained drug release capabilities. Additionally, chitosan's susceptibility to microbial degradation in the colon makes it an advantageous carrier for colon targeting (Kurakula et al., 2021). Matrix systems are frequently not sufficient to avoid drug release in the upper parts of the gastrointestinal tract. Film-forming polymers can achieve controlled drug release when they are applied to classic matrix tablets (Colombo et al., 2000; Maroni et al., 2013). Polymers derived from methyl acrylate esters, such as Eudragit®, are film-forming polymers whose chemical structure can be modified to, for example, resist degradation at low pH levels. When applied to tablets, these functional polymers, individually or combined, can achieve controlled drug release (Moustafine et al., 2012; Gupta et al., 2015; Borges Dos Reis et al., 2016; Arévalo-Pérez et al., 2020). Given the greater reliability of multiple drug release strategies in the colon (Sardou et al., 2024), this study aimed to use a combined approach involving an HPMC/chitosan matrix system film-coated with either a pH-dependent release polymer (Eudragit® RS 30D) or a time-dependent insoluble and highly permeable release polymer (Eudragit® RL 30D). The first one was pretended to avoid early release; Eudragit® RS 30D dissolves at a pH higher than 7, thus occurring when the formulation achieves the distal ileum (Fallingborg, 1999). The second one was designed to allow early entry of the physiological medium, thereby facilitating the diffusion of the drug from the matrix core when the formulation reached the colon, which has a low water content. Both formulations combine the functional coating with a sustained release matrix system in the core. The hydrophilic matrix core pretends to facilitate sustained release in the colon. This is due to the fecal stasis, fecal impaction, and trapping of feces in a diverticular sac, which may also result in infection, a characteristic feature of diverticulitis (Singh et al., 2016; Tursi et al., 2020).

The direct compression method, known for its simplicity, robustness, and cost-effectiveness, is the preferred approach for obtaining matrix tablets at an industrial scale. The simplicity of this two-step process has made it a perfect candidate to obtain tablets in an industrial way (Recife et al., 2017). However, despite the simplicity of the direct compression process, there are still numerous variables that require careful control to ensure the quality of the tablets (Thoorens et al., 2014), like formulation factors derived from the rheological characteristics of raw materials. Similarly, coating processes introduce various adjustable variables essential for obtaining both effective functional films and pleasing appearance for patients (Pandey et al., 2014).

Adopting the Quality by Design (QbD) methodology establishes a predefined workspace to attain a design space where the product's quality aligns with the initially described Quality Target Product Profile (QTPP) (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2009). This systematic methodology will allow us to determine which formulation factors lead to a drug release behavior in line with the stated objectives and to test whether the proposed formulation options would achieve a majority release of metronidazole in the colon.

Thus, the main objective of this work was to develop a metronidazole drug delivery system that would prevent high premature drug release in the upper GIT and achieve maximum drug concentrations at the infection site for the treatment of diverticular disease in the colon. This was achieved through the application of a QbD approach. The work will involve defining a QTPP, conducting a comprehensive Risk Analysis and evaluation, and a suitable Design of Experiments (DoE) to establish an appropriate Design Space. This framework will serve as a guide for the manufacturing of metronidazole-coated matrix tablets, ensuring maximum drug release in the colon.

2. Materials and methods

2.1. Materials

Metronidazole was purchased from Fagron Ibérica, Spain. HPMC 15 K was donated by Colorcon Ibérica (Spain), and HPMC 35 K of Ashland was donated by Saffic Alcan. Chitosan (CH) 1000–2000 cps; 1500,000 (avg.) molecular weight; degree of deacetylation $\geq$ 90.0 %, was obtained from Glentham Life Science. Colloidal dioxide silica, Aerosil® 200 VV Pharma, was donated by Evonik Spain and Glyceryl Behenate was donated by Gattefossé Spain. Polysorbate 80 and Glyceryl monostearate (GMS) was purchased from Acofarma (Spain). Triethyl citrate

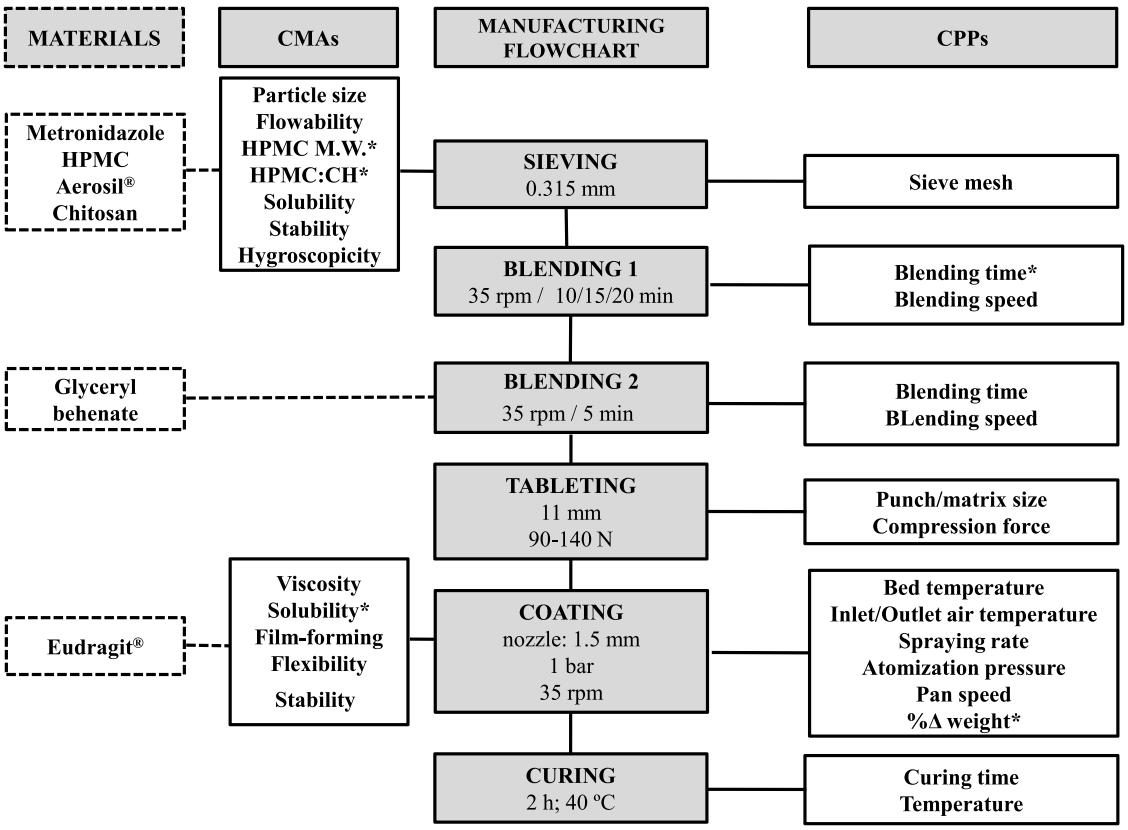

Fig. 1. Flowchart diagram for metronidazole coated matrix tablets. CMAs: Critical Materials Attributes; CPPs: Critical process Parameters; M.W.: molecular weight; *: CMAs and CPPs studied in the DoE.

Table 3
Rheological characterization of raw materials.

	Metronidazole	HPMC 35K	HPMC 15K	Chitosan
Flow time (s) ±SD	NA	NA	5.27 ± 0.25 *E*+00	11.50 ± 1.35 *E*+00
Bulk density (g/mL) ±SD	0.66 ± 3.23E-02	0.30 ± 5.81E-03	0.31 ± 3.54E-03	0.21 ± 1.36E-02
Tapped density (g/mL) ±SD	0.80 ± 4.04E-02	0.45 ± 6.10E-03	0.46 ± 3.11E-02	0.31 ± 3.54E-03
Carr Index (%) ±SD	17.63 ± 1.62E-01	32.60 ± 1.41E+00	32.12 ± 3.67E+00	30.37 ± 3.60E+00
Hausner Ratio ±SD	1.21 ± 2.39E-03	1.48 ± 3.10E-02	1.48 ± 8.19E-02	1.44 ± 7.22E-02
Angle of repose (°) ±SD	NA	NA	26.10 ± 8.47E-01	26.24 ± 2.95E+00
Moisture content (%) ±SD	0.20 ± 1.59E-01	3.36 ± 6.37E-02	3.13 ± 1.15E-02	6.34 ± 1.61E-01

NA = 100 g of powder do not flow through the funnel.

from Acros Organics was purchased from Fisher Scientific. Finally, Eudragit® FS30D, Eudragit® RL30D and PlasACRYL® T20 were received as samples generously donated by Evonik Spain.

2.2. Characterization of raw materials

Metronidazole particle size was determined with a sieving system with decreasing nominal mesh apertures employing sieves of 100 μm, 200 μm, 315 μm, 710 μm and 1.25 mm connected to the AR-402 All-Purpose system from Erweka® GmbH (Langen, Germany). To comprehensively characterize both the drug and excipients, bulk density and tapped density were investigated utilizing a tapped density tester SVM 102 (Erweka® GmbH, Langen, Germany), while flowability was assessed with a flow tester GT (Erweka® GmbH, Langen, Germany). Residual humidity data were obtained through Karl-Fischer titration on an 870 KF titrino plus (Metrohm® Hispania, Madrid, Spain) using three samples of 100 mg each.

The compatibility of metronidazole with pharmaceutical excipients was explored using Differential Scanning Calorimetry (DSC) on a DSC-1 (Mettler-Toledo S.A.E, Barcelona, Spain). Individual ingredients and binary mixtures (1:1) of metronidazole and pharmaceutical excipients were tested. Samples were weighed into 40 μm aluminium pans and heated at 10 °C/min from 25 °C to 250 °C. Thermal curve analysis was conducted using the STARe software package (Mettler-Toledo S.A.E, Barcelona, Spain).

2.3. QbD approach

2.3.1. Quality target product profile (QTPP)

The relevant specific requisites of the developed colon-controlled release coated tablets were included (Lionberger et al., 2008; International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2009; Hales et al., 2017). QTPP for the formulated tablets can briefly be described as follows: 500 mg metronidazole colon-controlled release coated tablets designed to achieve maximum dosage release in the colonic region 6 h post-ingestion. These tablets are coated with either, a gastric-resistant film or an insoluble and permeable film, pretending to ensure most drug release in the colon. The goal is to release the maximum drug content precisely at the infection focal point, thereby averting systemic distribution and minimizing potential side effects. For this formulation, a clear and concise definition of the QTPP, has been made.

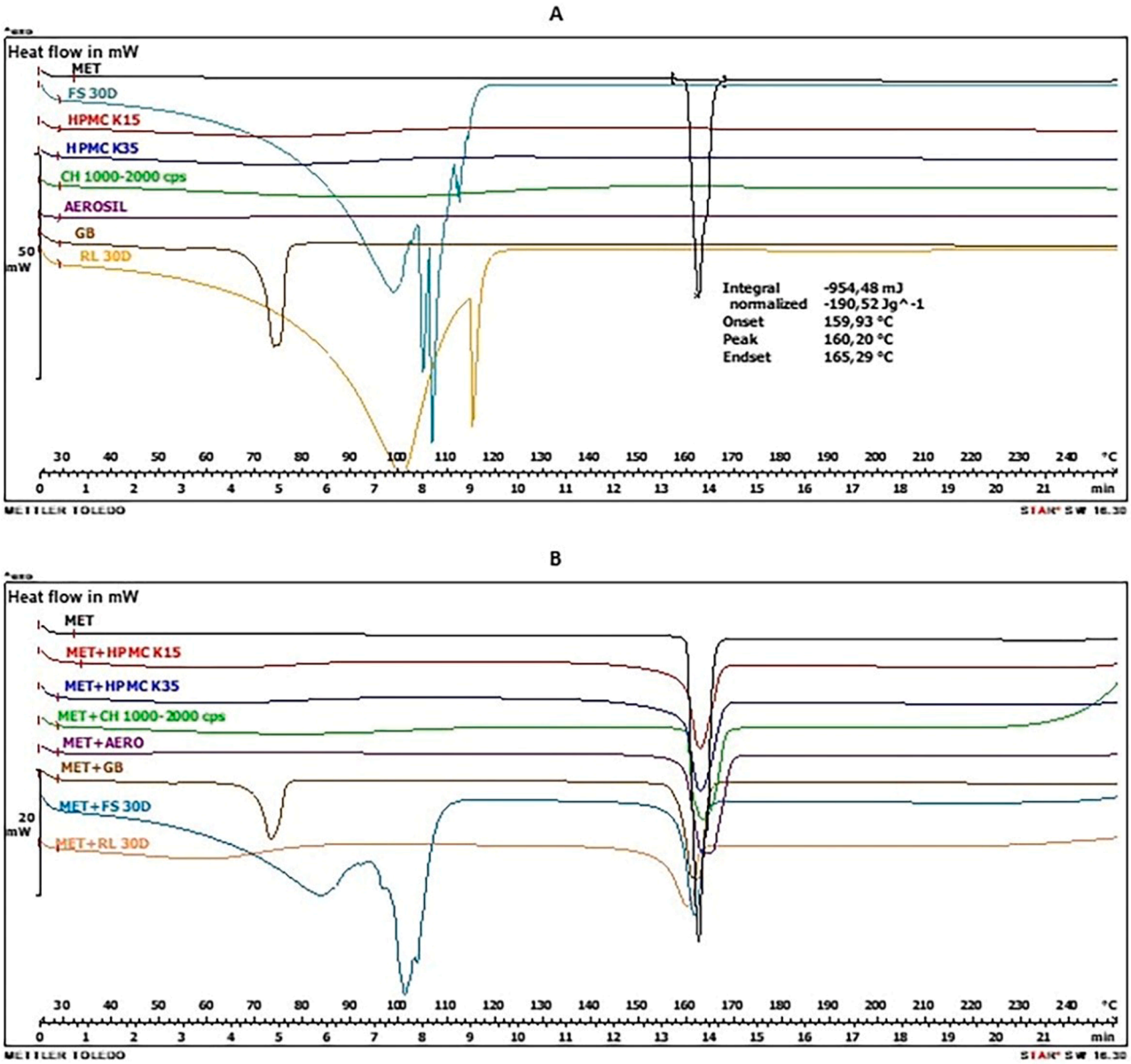

Fig. 2. DSC analysis of each individual component (A) and API + excipient mixtures (B). MET = metronidazole; CH = Chitosan; AERO = Aerosil®; GB = Glyceryl behenate; FS30 D and RL30D = Eudragit®.

2.3.2. Critical quality attributes, critical material attributes and critical process parameters (CQA, CMA and CPP)

The CQAs of the final product were identified through a comprehensive assessment, based on existing knowledge from the literature and previous experience. An Impact and Severity Analysis was carried out to determine if any material property and/or process parameter affected the QTPP (Waghule et al., 2021). The CQAs identified as having a significant impact following this analysis were subsequently incorporated into a thorough Risk analysis and Risk evaluation.

2.3.3. Risk analysis and Risk evaluation

An Ishikawa diagram was constructed to systematically outline potential causes of problems related to the final product, facilitating their inclusion in the Risk analysis. Subsequently, a Risk analysis was conducted to identify which CMAs and CPPs were associated with a high-risk of impacting CQAs.

A Risk evaluation was performed to quantitatively assess the identified risks. Utilizing a Failure Mode and Effects Analysis (FMEA) approach, a 1–5 scale was employed (refer to Tables S1-S3) (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2014). A score of 1 indicates a less likely risk with low severity and high detectability, while a score of 5 represents a very high probability and severity risk with low detectability. The Risk Priority Number (RPN) was obtained by multiplying the Probability (P), Severity (S) and Detectability (D) values as shown in Eq. (1):

$$RPN = PxSxD \quad (1)$$

The critical score considered for the Design of Experiments (DoE) to establish the Design Space were those with an RPN value exceeding 30. An RPN range of 30–40 denoted a medium risk (yellow), and values exceeding 40 indicated a high risk (red). CMAs and CPPs with RPN values below 30 (green) were objectively deemed to pose no significant risk to the QTPP and therefore required no further investigation.

Table 4
Quality Target Product Profile (QTPP) describing the characteristics of metronidazole colonic controlled release coated tablets.

CQA	Goal	Justification
Pharmaceutical form	Controlled-release coated tablet	Outperform the conditions of the upper GI
Pharmaceutical processes	Direct compression & coating	Easiest and most cost-effective development processes
Appearance	White round smooth coated tablets	Appealing look to facilitate patient compliance
Formulation design	Colonic control release	Slow and continuous drug release at the focal point
Administration method	Oral	Best patient compliance route of administration
Dose unity	500 mg	Minimum metronidazole dose effective against the disease
Pharmacokinetics	Control drug release up to 6 h (< 20 % drug release)	Release the maximum amount of drug in the colonic region
Product Quality Attributes	European Pharmacopoeia	–

Table 5
Defined CQAs for metronidazole coated matrix tablets.

CQA	Goal	Impact Analysis[1]	Severity Analysis[2]	Justification
Physical attributes	White, round	No	No	Ensure patient acceptance
	Film coated	Yes	No	Ensure tablet integrity and drug delivery to the colon
Size	11 mm diameter	No	Yes	Size and shape adequate to swallowing
Dosage	500 mg	No	Yes	Indivisible, avoids coating rupture
Blend Assay	90 – 110 %	Yes	Yes	European Pharmacopeia values
Blend U.C.	CV < 15 %	Yes	Yes	All batches should have the same amount of drug
Core tablet hardness	90 – 140 N	Yes	Yes	Directly affects drug release and mechanical stability of the tablets
Friability	< 1 % weight loss	No	Yes	Ensure tablet integrity
Dissolution	<20 % before 6 h ≈ 50 % at 24 h	Yes	Yes	Avoids early dissolution and gains sustained release
R.H.	<5 %	Yes	Yes	Avoid degradation and microbiological growth

U.C. = uniformity of content; CV: coefficient of variation, R.H: Residual Humidity.
[1] = Does any property of the materials and/or process parameter negatively affect the quality of the CQA described?
[2] = Does efficacy or/and safety of the final product compromised if the CQA fails to meet the specifications?

2.4. Design of experiments, DOE

A design of experiments (DOE) was carried out based on a fractional factorial design. This type of design allows the number of experiments to be reduced when the number of factors is high. On the other hand, this type of design allows for the inclusion of both continuous and non-continuous factors and their interactions, based on the Sparsity of Effects principle, which considers only a few main effects and low-order interactions influencing the system (McLean and Anderson, 1984). A 2^5 fractional factorial design was implemented, incorporating the insights gained from the FMEA analysis. The five factors resulting from the FMEA analysis were systematically considered at two levels (Table 1).

Three replicates are considered for each combination of factors to achieve consistent results. DoE was performed using the statistical software Minitab®18 (Minitab, LLC, USA) resulting in a total of 28 experiments or batches (Table 2).

2.5. Manufacture of tablet cores

Batches of matrix tablets of 750 mg weight were developed following a designed process outlined in the flow diagram (Fig. 1), with corresponding CMAs and CPPs. All matrix tablets consisted of 66.69 % metronidazole, 0.50 % Aerosil® 200 VV Pharma and 1.00 % glyceryl behenate. The remaining 31.81 % was apportioned among HPMC and CH in various ratios (3:1, 1:3, and 1:1) based on the specific batch described in the DoE.

Prior to the blending process, all components underwent sieving through a 0.315 mm sieve. Subsequently, blending was conducted using a biconic rotating drum from Erweka® GmbH (Langen, Germany) at a speed of 35 rpm for durations of 10, 15, or 20 min, depending on the batch specifications outlined in the DoE. Following an initial mix, the lubricant was added, and the blend underwent an additional 5 min of blending.

The resulting blend was subject to a direct compression process using a tablet compression machine (Model BMT®, Bonals, Spain) equipped with 11 mm round punches (Metalúrgica Lurga®, Lda, Bobadela, Portugal). The applied force during compression was carefully controlled to produce matrix tablets within a hardness range of 90 - 140 N.

2.6. Elaboration of coating dispersions and coating process

For the coating process, two different types of polymers were used, either a pH-dependent polymer (Eudragit® FS30D) or a time-dependent polymer (Eudragit® RL30D). An Eudragit® FS30D suspension was prepared by stirring the Eudragit® dispersion and water into the PlasACRYL® T20 suspension for 10 min, using a Heidolph RZR 2102 control agitator (Heidolph Instruments GmbH, Schwabach, Germany). The resulting spray suspension was sieved through a 0.315 μm sieve (Erweka® GmbH (Langen, Germany)). To prepare the Eudragit® RL30D suspension, water was heated to 70–80 °C, and then a solution containing polysorbate 80, triethyl citrate, and GMS was added and homogenized using the Heidolph RZR 2102 control agitator for 10 min. Subsequently, water was added to the hot GMS emulsion, and the mixture was cooled down to room temperature with continuous stirring. The resulting suspension was slowly poured into the Eudragit® RL30D dispersion while gently stirring. The final suspension was sieved through the 0.315 μm sieve.

Tablets were coated in an Erweka® DKE/DKS coating pan (Langen, Germany) equipped with a non-perforated stainless-steel pan at a rotation speed of 35 rpm. Coating was performed by spraying the coating suspension over a bed of tablets using a Dexter® Airsoft gun with a 1.5 mm nozzle and a spray air pressure of 1 bar, assisted by a heat focus to achieve a bed air temperature of 40 °C. The coated tablets underwent a curing process and were placed in a Nahita® 632/7 oven at a temperature of 40 °C for 2 h.

2.7. Blend characterization

The rheological study of the blend was carried out employing 100 g of the final blend. Dimensional parameters, including bulk density and tapped density, as well as Compressibility Parameters (Carr's index), were determined using an Erweka SVM® tapped density tester (Langen, Germany). Flowability parameters, encompassing the Hausner ratio and angle of repose, were assessed using an Erweka GT® flow tester (Langen, Germany). Residual humidity was obtained by weighing three samples of 100 mg of the blend and conducting a Karl-Fischer test on an 870 KF®

Table 6
Risk Evaluation categorizing RPNs associated with the materials used in the formulation.

Material	CMA	Justification*	P	S	D	RPN
Metronidazole	Particle size	Small particle sizes promote the appearance of electrostatic charges between particles and the machinery. Bigger particle sizes could compromise metronidazole dissolution.	2	4	3	24
	Rheology	Poor hopper emptying causes deficient matrix filling and uniformity of mass and hardness values out of specifications. Poor rheological characteristics (Table 3) are easily avoided by addition of a glidant to the formulation.	2	4	3	24
	Solubility	Metronidazole water solubility is low (10 mg/mL at 20 °C). Low water content in the colon complicates metronidazole dissolution.	3	4	4	48
	Stability	Photosensitivity: Metronidazole darkens in contact with light. Good manufacturing processes and an opaque container are enough to prevent darkening.	2	2	2	8
HPMC	Particle size	Small particle sizes promote the appearance of electrostatic charges between particles and the compression machine. Adding a lubricant easily solves this problem.	2	4	3	24
	Rheology	Poor hopper emptying causes deficient matrix filling, resulting in tablets with different proportions of API. Poor rheological characteristics (Table 3) affect uniformity of mass and/or tablet hardness. Poor feeding flow is easily avoided by addition of a glidant to the formulation.	2	4	2	16
	Hygroscopicity	Hydrophilic polymers easily capture water from the surroundings, which can affect tablet hardness and final tablet appearance. Lipophilic lubricants can minimize the risk.	2	3	2	12
	Molecular Weight (MW)	Drug release is strongly affected by HPMC´s molecular weight. Low MW allows undesired early drug release, whereas high MW could avoid enough drug release (Jain et al. 2014)	5	4	4	80
Chitosan	Rheology	Poor hopper emptying causes deficient matrix filling, resulting in tablets with different proportions of API and affects uniformity of mass and/or tablet hardness. Poor rheological characteristics (Table 3) are easily avoided by addition of a glidant to the formulation	2	4	2	16
	Solubility/ Swelling	Chitosan cannot form strong gel layers by itself, and it is soluble under acidic conditions (pKa 6.5) (Arévalo-Pérez et al. 2020). Different chitosan proportions in the formulation can modify drug release.	4	4	3	48
	Hygroscopicity	Hydrophilic polymers easily capture water from the surroundings, which can affect tablet hardness and final tablet appearance. Lipophilic lubricants can minimize the risk.	2	3	2	12
Eudragit® coating agents	Composition	Polymethacrylates´ varying ratio composition can affect its functionality, turning into pH-dependent or time-dependent polymers which affect drug release mechanisms	4	5	2	40
	Solubility	pH-dependent polymers only dissolve above a certain pH (pH 7.0 in colon delivery systems) Time-dependent polymers are water insoluble. Coating weigh gain will determine coating strength.	5	5	2	50
	Flexibility	Methacrylate polymers form easily breakable rigid films. Adding a plasticizer reduces the risk	3	3	3	27
	Film forming	Eudragits® are known to form excellent film layers.	2	4	3	24
	Stability	Eudragits® are stable and do not degrade if the immediate or outer packaging is appropriate.	2	3	2	12

* Supporting quantitative data shown in Y0 (Szymańska and Winnicka, 2015; Quinn et al., 2016; Zhang et al., 2018). *Green = RPN`s with low risk, Yellow = RPN`s between 30 – 40 associated with moderate risk; Red = RPN`s above 40 indicating high risk. P= Probability; S= Severity; D=Detectability; RPN= Risk Priority Number; M.W. = Molecular weight.*

Table 7
Risk Evaluation categorizing RPN associated with the process.

Process step	CPP	Justification*	P	S	D	RPN
Blending	Blending time	Short/long blending times generate mixtures with low quality levels and bad content uniformity.	3	4	4	48
	Blending speed	Alterations in rotation speeds could lead to blend segregation.	3	4	4	48
Compression	Punch size	Punch and matrix size should be adequate to produce easily swallowed tablets.	1	2	2	4
	Compression force	Different compression forces affect tablet hardness and dissolution rates. Machine adjusted compression force is robust.	2	3	2	12
Coating process	Air Temperature	Low bed T not high enough to evaporate coating dispersion solvents might produce sticking problems. High bed T° avoids adequate film formation, and defects such as wrinkles, cracks and excessive roughness can appear.	3	4	3	36
	Spray rate	Low spray rates could form inadequate films and produce unappealing spotted tablets. High spray rates can over-wet tablets, soaking the pan loaded bed, producing sticking and twinning phenomena.	3	4	3	36
	Atomization Pressure	Atomization pressure must be high enough to produce fine droplets independently of the coating dispersion viscosity.	3	4	3	36
	Pan Speed	Slow pan speed can produce sticking phenomena as tablets could be under the atomized dispersion longer time, meanwhile fast pan speed could prevent enough coating dispersion from being laid down onto tablets.	3	4	3	36
Curing	Time	Prolonged curing times could affect films permeability.	2	3	2	12
	Temperature	Increasing temperatures can modify film assembly, increasing release rates.	2	3	2	12

* Supporting quantitative data shown in Table S11 (Agrawal and Pandey, 2015).
Green = RPN`s with low risk, Yellow = RPN`s between 30 – 40 associated with moderate risk; Red = RPN`s above 40 indicating high risk. *P*= Probability; S= Severity; D=Detectability; RPN= Risk Priority Number.

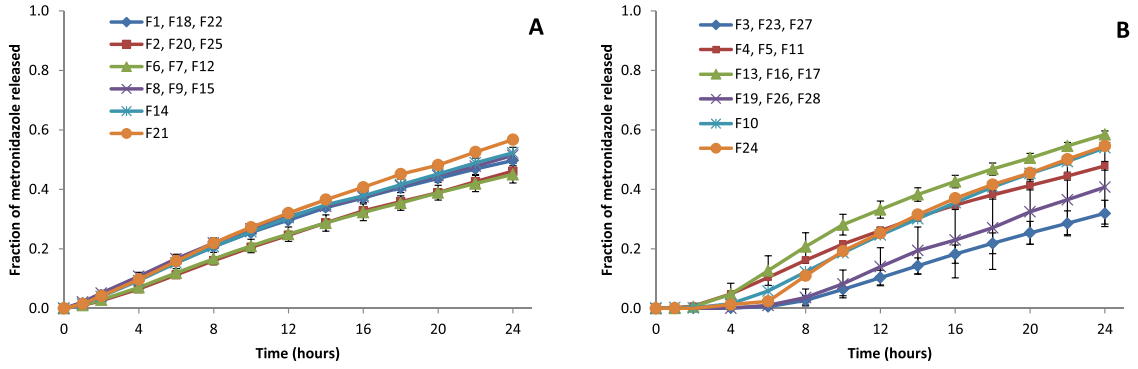

Fig. 3. Mean release profiles from three replicates and all four central points. (A) Batches coated with Eudragit® RL 30D (Time-dependent coating); (B) Batches coated with Eudragit® FS 30D (pH-dependent coating).

titrino plus (Metrohm® Hispania, Madrid, Spain).

The assay and uniformity of content of the final blend were analyzed based on a stratified sampling of 375 mg. A total of 6 samples per batch were taken, and Metronidazole concentration was determined using a UV–visible Agilent 8453® spectrophotometer (Agilent, USA), considering a wavelength λ = 320 nm, and a 1 mm path length.

2.8. Tablets characterization

Uncoated core tablets were evaluated by the following tests: Uniformity of mass was performed by weighing 20 tablets on an analytical balance Mettler® XS-105 DU (Mettler Toledo, USA). Tests of tablet thickness, tablet diameter, and hardness were performed on a total of 10 tablets on an Erweka TBH 210TD® Hardness tester (Erweka, Langen, Germany). The Pharmacopoea friability test was done using an Erweka

Table 8
Mean kinetic parameter values from the Weibull model out of every three replicates and all four central points.

		Weibull			
	Batches	Fmax ± SD	MDT (h) ± SD	β ± SD	t_{lag} (h) ± SD
Batches coated with Eudragit® RL 30D	F1, F18, F22	0.95 ± 4.51E-01	28.74 ± 5.64E+00	1.02 ± 2.73E-01	0.89 ± 1.13E+00
	F8, F9, F15	0.97 ± 1.02E-01	26.17 ± 1.77E+00	0.94 ± 3.59E-02	0.59 ± 2.71E-01
	F6, F7, F12	0.90 ± 1.84E-01	40.30 ± 1.50E+01	1.05 ± 1.52E-01	0.89 ± 4.32E-01
	F2, F20, F25	1.01 ± 1.69E-01	38.31 ± 6.21E+00	1.01 ± 7.66E-02	1.54 ± 5.36E-01
	F21	1.11	24.10	0.86	2.03
	F14	1.30	32.01	0.85	1.36
Batches coated with Eudragit® FS 30D	F4, F5, F11	0.88 ± 2.12E-01	22.47 ± 2.49E+00	0.95 ± 1.34E-01	1.89 ± 9.51E-01
	F13, F16, F17	1.05 ± 3.01E-01	15.93 ± 1.29E+00	0.86 ± 1.41E-01	3.50 ± 1.81E+00
	F3, F23, F27	1.00 ± 2.79E-01	55.45 ± 2.15E+01	1.07 ± 1.00E-01	6.73 ± 1.53E+00
	F19, F26, F28	1.05 ± 5.66E-01	48.98 ± 2.10E+01	1.15 ± 3.16E-01	6.86 ± 1.73E+00
	F24	0.93	19.22	0.91	5.62
	F10	1.04	30.88	0.99	4.47

$F = Fmax * \left(1 - exp\left[\left(-\frac{(t - t_{lag})^{\beta}}{MDT}\right)\right]\right)$; Fmax = maximum fraction of the dose dissolved; MDT = mean dissolution time; β = shape fitting parameter of Weibull equation; t_{lag} = lag time period; SD= Standard Deviation.

TAR 100® friability tester (Erweka, Langen, Germany). Finally, residual humidity was obtained from 10 pulverized tablets, weighing three samples of 100 mg of the resulting powder, and carrying out a Karl-Fischer test on an 870 KF® Titrino Plus (Metrohm® Hispania, Madrid, Spain). The same tests were performed on coated tablets except for the friability test.

2.9. Dissolution testing

A dissolution test was carried out on 6 coated tablets out of every batch developed using a qualified DT808® Dissolution tester (Erweka, Langen, Germany) with paddles (USP Method II) at a rotation speed of 50 rpm for 24 h in 900 mL of dissolution phosphate buffer at pH 7.2 and temperature maintained at 37 °C ± 0.5 °C. Previous disintegration tests for the worst case, 10 % coating weight gain formulations coated with the two polymers (FS & RL), showed resistance to the acidic medium. Therefore, dissolution tests were performed without it. Sampling was performed at the first hour and then every two hours through 24 h, 1 mL per sample. Metronidazole concentration was determined using a UV-visible Agilent® 8453 spectrophotometer (Agilent, USA), considering a wavelength λ = 320 nm, and a 1 mm path length. Dissolution testing was also carried out on the free drug.

A discriminative test (similarity factor or *f*2 calculation) has been performed to confirm the validity of the dissolution method. Drug release data from two formulations with pH-dependent coating and another two formulations with time-dependent coating were used to calculate *f*2.

To test the complete release profile, two worst-case scenarios have been considered using the slowest release profile for each of both coating polymers: Eudragit RL 30D (batch F7) and Eudragit FS 30D (batch F23).

2.10. Kinetic analysis

Mean release dissolution profiles obtained *in vitro* were fitted to define the best model that describes the drug release behavior from the matrix tablets. A set of four models was proposed for the kinetic analysis of the drug release profile curves: Zero-order, First-order, Korsmeyer-Peppas and Weibull. The curve fitting of each dissolution curve was performed by weighted non-linear regression (Yamaoka et al., 1981). Akaike's information criterion was considered a relative criterion of goodness of fit.

2.11. Statistical analysis and design space establishment

In this study, a single design space was created that would allow the development of a product with the desired QTPP. Although two DoEs could have been made, one for the core and the other for the coating, it has been preferred to work with a single DoE that includes the matrix core and the coating to simplify the experimental design. Moreover, a combined DoE including core and coating allows for the study interactions between core and coating factors. Using the Minitab®18 statistical software (Minitab, LLC, USA), an ANOVA regression was conducted, incorporating all factors from the DoE individually as well as exploring potential interactions among them. Factors exclusively affecting coated tablets, such as the type of coating polymer and coat weight increase, were excluded from the factorial analysis focused on variables responsive to the core tablets. If one or more factors demonstrated a statistically significant influence on the response variables, a 'backwards' elimination procedure was executed to remove non-significant factors from the analysis. The results obtained from the statistical analysis led to the design space.

3. Results and discussion

3.1. Characterization of raw materials

Table 3 shows the results of the raw material characterization. Poor rheological characteristics were obtained for the raw materials tested. These data are considered later in order to make decisions over risk factors management (Table 6).

Fig. 2 show the results obtained for the compatibility study by DSC. DSC curves obtained from each individual component are shown in Fig. 2A and curves obtained from API and excipient binary mixtures are shown in Fig. 2B. Compatibility thermograms do not show interactions between metronidazole and the tested excipients, since the melting point of the drug in binary mixtures occurs at the same temperature as the single metronidazole curve.

3.2. QbD approach

Table 4 gathers all the information folded into the QTPP of the developed formulation. Table 5 shows the Impact and Severity Analysis carried out to choose the CQAs studied in the Risk Analysis and Risk Evaluation.

All steps of the development process were listed in an Ishikawa Diagram (Fig. S1) as previous process to the Risk Analysis to determine the potential causes of problems related to metronidazole coated tablets development.

Risk analysis results (Tables S4-S9) show which CMAs and CPPs were involved in CQAs.

Table 6 shows the risk evaluation of the materials used in the preparation of the colonic coated matrix tablets of metronidazole.

Table 7 shows the risk evaluation of the processes used in the preparation of the colonic coated matrix tablets of metronidazole.

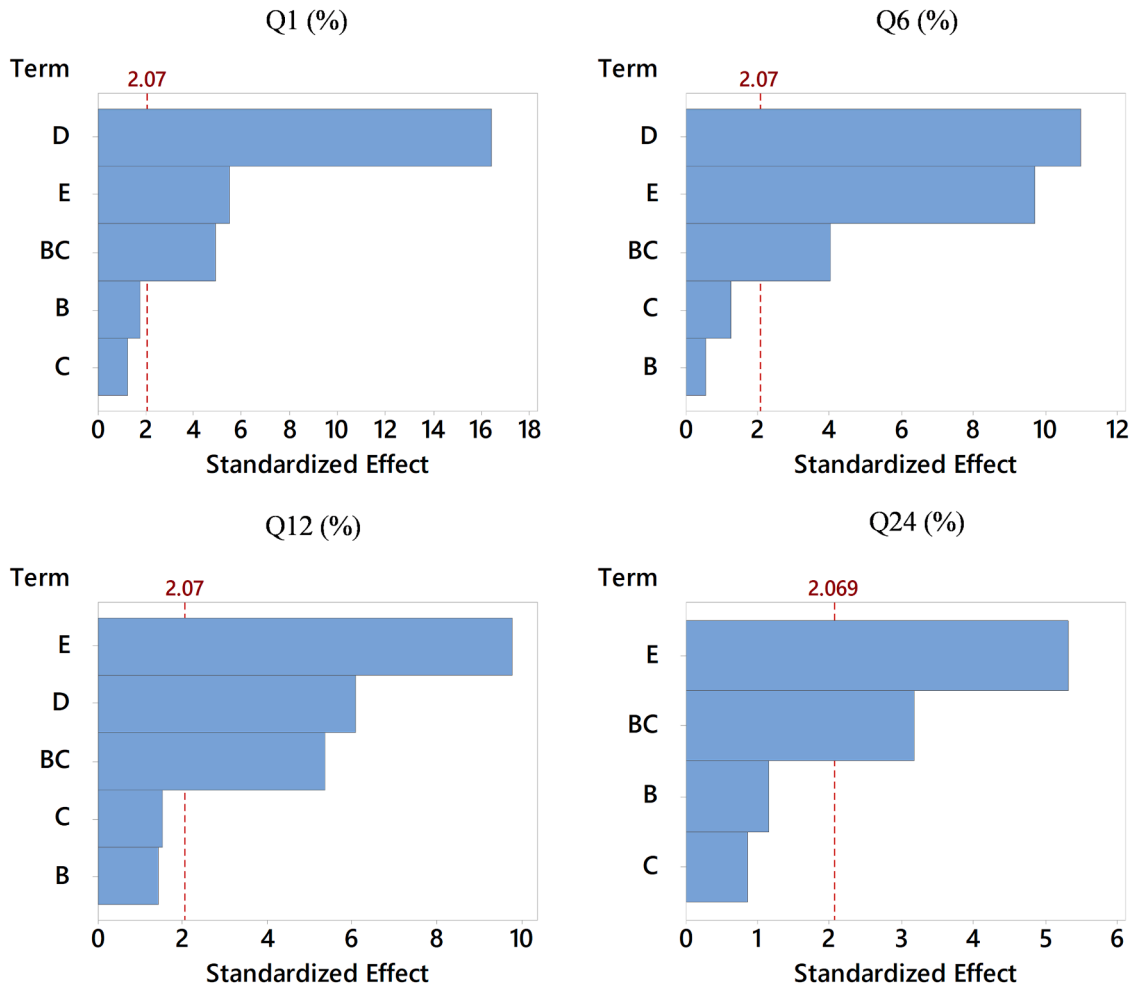

Fig. 4. Pareto charts of standardized effects on metronidazole released at the first hour, 6, 12 and 24 h post-backwards elimination. α = 0.05. Terms: B = HPMC/CH ratio, C = Blending time, D = Coating agent, E = Total increase on weight after coating.

3.3. Blend and tablets characterization

Blends pre-compaction demonstrated reproducible rheological characteristics (Table S12). Additionally, both the core tablets (Table S13) and coated tablets (Table S14) from all manufactured batches exhibited consistent and reproducible technological characteristics. This suggests a high degree of reproducibility in the manufacturing process, even when batches are produced under different conditions.

Hardness values exceeding 140 N (Table S13) are the consequence of the adjustment of the compression force on the tableting machine or the plastic behavior of Eudragit® RL 30D (Abbaspour et al., 2007; Gupta et al., 2015). Coated tablets with Eudragit® RL 30D deform without breaking, which the durometer interprets as extremely high hardness values (Table S14).

3.4. Dissolution tests

The dissolution tests have provided valuable insights into the release profiles, showcasing the distinct behavior of the developed tablets. The utilization of different coating polymers has resulted in two well-differentiated release profiles, as illustrated in Fig. 3. Time-dependent coated tablets released their content from the first hour (Fig. 3A). On the other hand, tablets coated with Eudragit® FS 30D (pH-dependent polymer) displayed a lag time period that increased with the percentage of the total weight increase after coating (Fig. 3B). The difference in the % coating of the pH-dependent polymer produces a variability on the release profiles that cannot be appreciated over the release profiles coated with the time-dependent polymer.

The dissolution method is considered discriminative when comparing profiles resulting in an *f2* value lower than 50 (Table S15). Free drug release data has been shown in Figure S2, and results of the complete release profile for both coating polymers are shown in Figure S3. The pH-dependent formulation (FS 30D) conducts a slower release for the first 24–36 h, but after 3 days, the time-dependent formulation (RL 30D) presents a slower release than the pH-dependent one. Both formulations have completed the release of metronidazole after 5–6 days.

3.5. Kinetic analysis

According to the goodness of fit criteria, the model selected to characterize the dissolution profiles of metronidazole was the Weibull model. Table 8 depicts the average values of the parameters of the Weibull equation for every three replicates and all four central points.

The Weibull model provides information about releasing rates in the terms mean dissolution time (MDT) and β constant related to the specific release mechanisms (Kosmidis and Macheras, 2007). The Mean Dissolution Time (MDT) shows to be considerably long, exceeding 24 h in the majority of the cases, which implies that a sustained drug release is achieved. However, MDT presents high values, up to 50 h in some cases (Table 8), which could limit the therapeutic effect as the majority of the dose would be expelled with the stools (Maity and Sa, 2016).

Although it presents the best fitting values, the Weibull equation is

Table 9
Regression equations for each variable studied with a statistically significant outcome after the backwards analysis.

	RESPONSE, units	REGRESSION EQUATION	R^2(%)
Blend	Uniformity of content (U.C.) (CV %)	U.C. = 2.121 - 0.869 *A* + 0.747 *B* + 0.730 A*B	31.00
	Flow (S)	Flow = 2.1111 – 0.0749 B	16.64
Cores	Residual Humidity (R.H.) (%)	R.H. = 2.966 – 0.1507 B – 0.0226 C	41.64
	Hardness (N)	Hardness = 118.36 + 13.06 *A* + 3.69 B - 4.87 A*B	31.54
	Diameter (mm)	Diameter = 11.0710 – 0.00749 B	19.77
Coated Tablets	Uniformity of mass (U.M.) (g)	U.W. = 0.7725 + 0.006608 E	79.47
	Residual Humidity (R.H.) (%)	R.H. = 4.080 – 0.1844 B	19.87
	Hardness (N)	Hardness = 110.7 – 74.9 *D* + 12.50 E	75.37
	Thickness (mm)	Thickness = 7.7598 + 0.03823 E	67.86
	Diameter (mm)	Diameter = 11.2165 + 0.03418 E	83.52
	Q1 (%)	Q1 = 0.777 + 0.554 *B* + 0.0435 C + 0.6771 D - 0.04900 E - 0.03296 B*C	91.82
	Q6 (%)	Q6 = 29.44 - 3.808 B - 0.506 C + 4.084 D - 0.7776 *E* + 0.2426 B*C	91.38
	Q12 (%)	Q12 = 59.43 – 8.27 B – 1.037 C + 3.540 D - 1.229 *E* + 0.5059 B * C	88.30
	Q24 (%)	Q24 = 84.03 – 9.39 B – 1.135 C – 1.250 *E* + 0.558 B * C	63.75

Q1, Q6, Q12 and Q24 are the amount of metronidazole released (%) at 1, 6, 12 and 24 h respectively. R2 (%) is the goodness of fit for each equation. Terms: A = HPMC grade (K15 = −1/K35 = +1), B = HPMC/CH ratio (0.33/3), C = Blending time (10/20 min), D = Coating agent (Eudragit® FS 30D = −1/Eudragit® RL30D = +1), E = Total increase on weight (10 %/20 %).

based on an empirical model, and it does not refer to the dissolution kinetics properties of the drug from the tablet cores. Correlations *in vitro/in vivo* can't be assumed, as there is no parameter describing the intrinsic dissolution factor of the drug (Mathematical models of drug release, 2015). However, some studies have shown a correlation between the power of time β and the drug release mechanisms from matrix tablets (Papadopoulou et al., 2006). Most of the curves in our study display β values either above 1, indicative of complex release mechanisms where the release rate does not exhibit constant change, or falling between 0.75–1, suggesting a drug release governed by diffusion with the contribution of another release mechanism. However, although all the β values are above or below 1, most of them are closer to 1 (Table 8). When β = 1, a first-order release can be assumed (Mathematical models of drug release, 2015). In addition, based on the Korsmeyer-Peppas model most cases studied showed *n* exponents with values between 0.45 and 0.89, fitting a non-fickian or anomalous release, meaning drug release is governed by two different processes (Shoaib et al., 2010).

Hydrophilic matrix tablets trigger drug release by quickly forming a gel layer when exposed to aqueous environments, allowing the drug to dissolve and diffuse through the gel layer.

This transport will depend on many internal and external factors, such as the viscosity of the hydrophilic polymers, their proportion in the formulation, the solubility of the drug, and the characteristics of the dissolution medium (Maderuelo et al., 2011; Jain et al., 2014; Mašková et al., 2020). In our studied batches, the combination of HPMC and CH, constituting less than 32 % of the tablet composition compared to the 66 % metronidazole, a low soluble drug (10 mg/mL) (Nasseh et al., 2019).

Table 8 also shows a significant increase in the lag time in batches coated with Eudragit® FS 30D (pH-dependent polymer), confirming what was observed in Fig. 3B.

3.6. Statistical analysis and design space establishment

In the ANOVA performed, the responses of the blend and core were initially considered independently, excluding coating-specific factors (Table S16). Backward elimination identified influential factors: HPMC/CH ratio, blending time and the interaction between the HPMC/CH ratio and HPMC grade (Fig. S4). Despite all factors having p values < 0.05, the R^2 values suggested suboptimal model fitting, especially for blend and core tablet characterization. An interaction between HPMC/CH ratio and blending time affecting drug release was observed, possibly due to suboptimal blending of hydrophilic polymers in the core of the matrix.

Subsequent ANOVA regression for coated tablets and the results obtained from the release profiles, including coating agent and weight gain, revealed these as factors affecting the characteristics of the coated tablets, which was corroborated by backward analysis (Fig. S5). R^2 values indicated improved model fit (Table S16).

Drug release was significantly influenced by the coating agent and weight gain. Although the use of coating polymers and weight gain are expected to affect metronidazole drug release, the use of the DoE methodology allows us to quantify the real influence of the coating on the performance of the film-coated colonic release matrices. In the case of Eudragit® FS 30D, a pH-dependent dissolution occurs and when Eudragit® RL 30D is used, an insoluble diffusion gel layer is generated, delaying release (Moustafine et al., 2012; Gupta et al., 2015; Hudovornik and Vrečer, 2015; Borges Dos Reis et al., 2016; Moghimipour et al., 2018). After 24 h, the coating agent had no impact on the response (Fig. 4). The interaction between hydrophilic polymers proportion and blending time significantly influenced metronidazole release at different intervals.

While R^2 values indicated a good fit, decreasing with testing time, this may be attributed to eliminated predictors in backward elimination (Table S16), still ensuring acceptable predictive accuracy.

In summary, there are several factors that statistically affect the mixture, the characteristics of the tablet core and the release profiles. These factors were identified as the HPMC/CH ratio, blending time, coating polymer, coating weight gain, interaction HPMC/CH ratio * blending time and interaction HPMC viscosity grade * HPMC/CH ratio.

Regression equations were established to predict responses when modifying these factors (Table 9). These equations effectively predict, among other variables, amounts of drug dissolved in response to factors impacting the percentage of drug released at 6 h and 24 h (Fig. 5).

Considering the QTPP proposed (Table 4), and because a solid object in a fasted state takes between 3 and 6 h to reach the colon under normal conditions (Abuhelwa et al., 2017), we focused our attention on what happened at drug release before 6 h.

Fig. 5A illustrates estimated responses at 6 h, showcasing formulations that meet the QTPP goal, except those with 10 % weight gain after coating with Eudragit® RL 30D, 8 % HPMC, and 10 min blending time (Fig. 5A red square). pH-dependent polymer-coated formulations release the least drug within 6 h, especially with 20 % weight gain and longer blending times at higher HPMC proportions.

Once the colon is reached, the objective of our formulations is to release metronidazole in a sustained way up to 24 h. Fig. 5B display the estimated responses of dissolution rate (%) at 24 h as a result of the factors HPMC, blending time, HPMC/CH ratio and % increase in total weight after coating.

After 24 h, HPMC viscosity grade and coating agent have no impact on drug release. Interaction between blending time and ratio HPMC/CH, along with the % increase in total weight after coating, influencing variability in the amount of metronidazole released at 24 h.

Batches with a 10 % weight increase and varied HPMC viscosity grades and coating polymers achieve ~50 % drug release. While transit time through the colon is inherently slow and variable, generally exceeding 20 h and potentially extending up to 42 h under specific conditions (Bak et al., 2018; Kotla et al., 2019), it is crucial to assess its implications for therapeutic efficacy. Batches with a 20 % weight increase along, with either high proportions of HPMC and short blending times or low proportions of HPMC and longer blending times, can retain almost 65 % of the dose of metronidazole at 24 h (Fig. 5B, red squares),

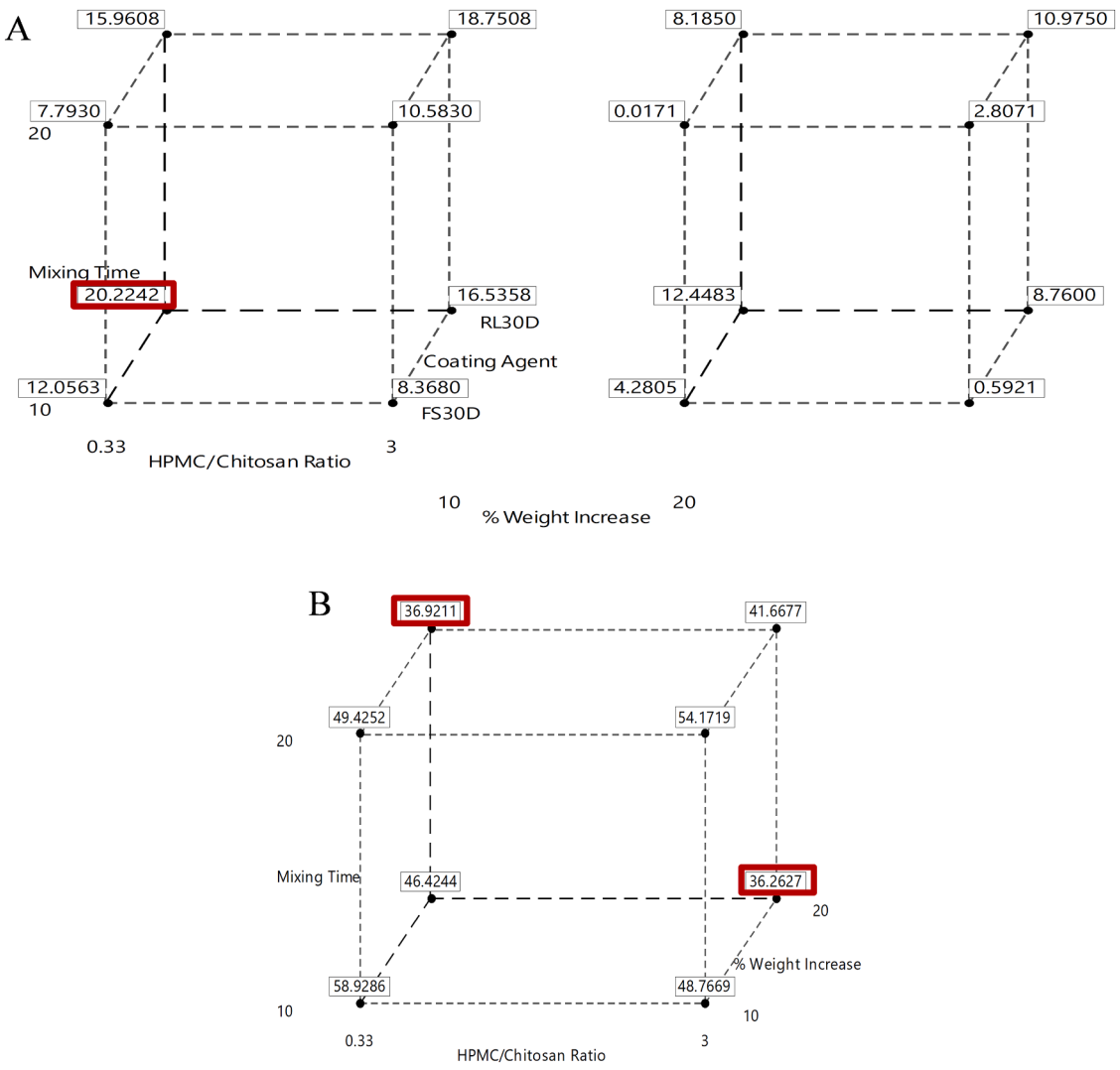

Fig. 5. Cube plots represent estimated fitted means of dissolution rate (%) at 6 h (5A), responding to HPMC, blending time, HPMC/CH ratio, coating agent (Eudragit® RL 30D or Eudragit® FS 30D) and % increase in total weight after coating and at 24 h (5B) responding to HPMC, blending time, HPMC/CH ratio and % increase in total weight after coating. Red square reflects the condition that doesn't achieve the goal proposed in the QTPP.

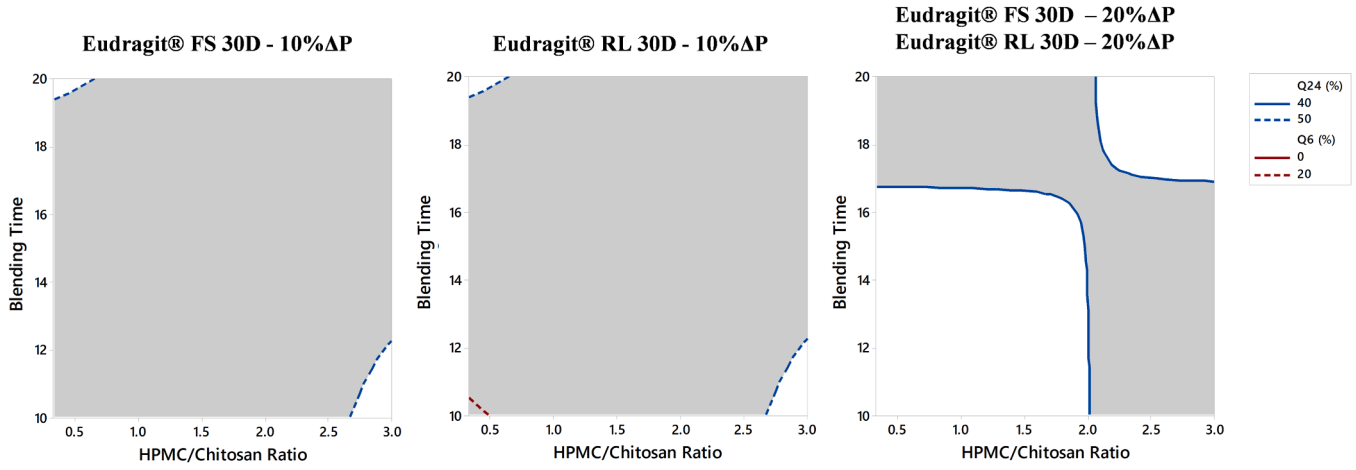

Fig. 6. Overlay contour plots for Q6 = 0–20 % and Q24 = 40–50 %. The design space is defined (unshaded) and represents combinations of the blending time and HPMC/chitosan achieving QTPP goals. Blue lines indicate limits below 40 % drug release at 24 h and the dotted red line represents limits below 20 % at 6 h.

may lead to therapeutic inefficacy, potentially ejected with stools.

However, altering the HPMC/CH ratio and blending time conditions, specifically opting for lower concentrations of HPMC with short blending times or higher proportions of HPMC with longer blending times, results in coated tablets with a 20 % increase in total weight after coating achieves metronidazole concentrations at 24 h between 40 and 50 %. While still relatively low, these outcomes are more reproducible when compared to other sustained-release formulations. Therefore, it is plausible to assume that pH-dependent coatings, particularly in higher proportions, can effectively target the liberation of metronidazole within the colon.

The unshaded area in Fig. 6 depicts the design space where experiments meet criteria. This Design Space, visualized in Fig. 6, aligns variations in factors impacting drug release with QTPP goals. In the case of formulations with a 10 % weight increase, Fig. 6 indicates the need to open and explore new levels of the critical factors to achieve the QTPP. For formulations with 20 % coating weight gain, the contour plot shows more possibilities of combinations of the factors studied that result in tablets that conform to the QTPP. Regression equations (Table 5) aid in selecting factor levels for desired responses within this Design Space, enhancing formulation predictability.

4. Conclusions

Based on a Quality by Design (QbD) methodology, a Quality Target Profile (QTPP) of metronidazole matrix tablets was developed. This system is capable of releasing the greatest possible amount of the dose in the colon to treat uncomplicated diverticulitis.

A hydrophilic matrix tablet was designed through a direct compression method, incorporating HPMC and chitosan as primary hydrophilic polymers. The core underwent a coating process with a functional polymer layer to govern drug release in either a pH-dependent or time-dependent manner.

Risk Management made it possible to select the factors that may have a potential impact on the defined QTTP. A design space was established that guarantees the objectives established in the QTPP. This space design is based on the interaction of HPMC/CH ratios with mixing times. The optimized design space allows the evaluation of variations in factors affecting metronidazole release according to the QTPP objectives. Within this Design Space, we can confidently operate, ensuring the compliance with the requirements established in the QTPP. The results obtained demonstrate that the QbD methodology constitutes a powerful tool for the design of controlled-release pharmaceutical dosage forms for oral administration such as colonic release formulations.

Compliance with ethical standards

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

CRediT authorship contribution statement

Roberto Arévalo-Pérez: Writing – original draft, Visualization, Resources, Methodology, Investigation, Formal analysis, Data curation. **Cristina Maderuelo:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Formal analysis, Conceptualization. **José M. Lanao:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Formal analysis, Conceptualization.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2024.106918.

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