1-1-2022

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Recommended Citation

Xu, Y., Yan, G., Wen, X., Wu, L., Deng, R., Liang, Q., Zhang, L., Chen, H., Feng, X., & He, J. (2022). Preparation, evaluation, and pharmacokinetics in beagle dogs of a taste-masked flunixin meglumine orally disintegrating tablet prepared using hot-melt extrusion technology and D-optimal mixture design. European Journal of Pharmaceutical Sciences, 168, 106019. https://doi.org/10.1016/j.ejps.2021.106019

European Journal of Pharmaceutical Sciences 168 (2022) 106019

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Preparation, evaluation, and pharmacokinetics in beagle dogs of a taste-masked flunixin meglumine orally disintegrating tablet prepared using hot-melt extrusion technology and D-optimal mixture design

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ARTICLE INFO

Keywords:
Flunixin meglumine
Hot-melt extrusion
Taste-masked
Orally disintegrating tablet
D-optimal mixture design

ABSTRACT

Flunixin meglumine (FM) is a nonsteroidal anti-inflammatory drug limited by irritation of the respiratory tract and mucosa in veterinary tissue. This study aimed to develop a taste-masked FM solid dispersion (SD) by hot-melt extrusion (HME) and formulate an orally disintegrating tablet (ODT) with selected excipients by direct compression. Eudragit® E PO was chosen as the matrix, and HME parameters were optimized: extrusion temperature, 135℃; screw speed, 100 rpm; and drug loading, 20%. Characterization techniques proved that FM was rendered amorphous in the HME extrudate. *In vitro* dissolution studies showed that FM SD released significantly slower than the corresponding physical mixture in artificial saliva. Excipients were selected based on compression formability, disintegration, and solubility. A D-optimal mixture design was used to optimize the composition: 25% FM SD, 18.75% microcrystalline cellulose, 52.5% mannitol, 3.75% low-substituted hydroxypropyl cellulose, and 1% magnesium stearate. Taste-masked FM ODT had a tensile strength of 0.7 ± 0.01 MPa and a disintegration time of 17.6 ± 0.1 s. E-tongue and E-nose analysis showed that FM ODT had a better taste-masked effect than commercial granules. Finally, a pharmacokinetic study proved that the main pharmacokinetic parameters of FM ODT were not significantly different from those of commercial granules, which indicated that these formulations had similar pharmacokinetic behaviours in beagles.

1. Introduction

With improvements in living standards, an increasing number of people are keeping pets, and this industry has been growing rapidly (Alves and Rocha, 2018). Pets have been continuously kept throughout history. Human–companion animal interactions afford physiological and psychosocial-like cardiovascular benefits and benefits for individuals with psychiatric disorders, nursing home residents, and children (Barker and Wolen, 2008; Poresky et al., 2016).

However, companion animals, especially older pets and racing animals, often suffer from arthritis, arthralgia and inflammation. These conditions undermine pet health and competitiveness levels. Nevertheless, there are only a few medications that can currently be used for arthritis.

FM is widely used for the treatment of dairy cow mastitis (Yeiser et al., 2012), pain management in the dehorning of calves (Huber et al., 2013), postoperative pain management in horses (Naylor et al., 2014), etc. However, it is limited by irritant effects, and most formulations are injection, granule, premix, and powder. An irritating taste will lead to poor adaptability and reduce feed intake and curative effects (Mair et al., 2010). Conventional injection solvents such as dimethylacetamide and dimethylformamide possess high toxicity, which may cause injury and even food safety issues (Weiner and Kotkoskie, 2000). Furthermore, normal injections result in rapid metabolism, which means higher administration frequencies and labour costs, especially in large and sensitive animals, causing stress responses and affecting treatment (Keyser et al., 2007). Hence, it is interesting to develop new formulations of FM to fully exploit its clinical value. Convenient medication can improve patient compliance, therefore improving the overall therapeutic index (Witchey-Lakshmanan and Li, 2000). The key for this

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https://doi.org/10.1016/j.ejps.2021.106019
Received 10 April 2021; Received in revised form 19 September 2021; Accepted 22 September 2021
Available online 24 September 2021

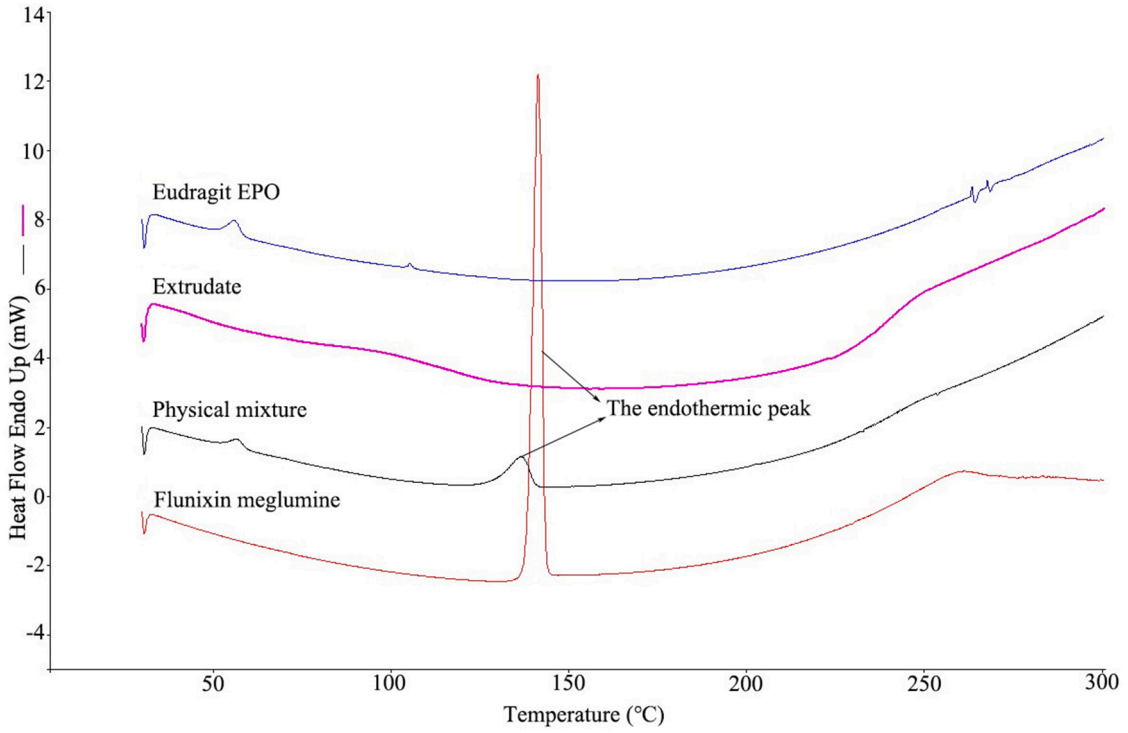


Fig. 1. DSC analysis of FM, Eudragit® E PO, physical mixture and extrudate.

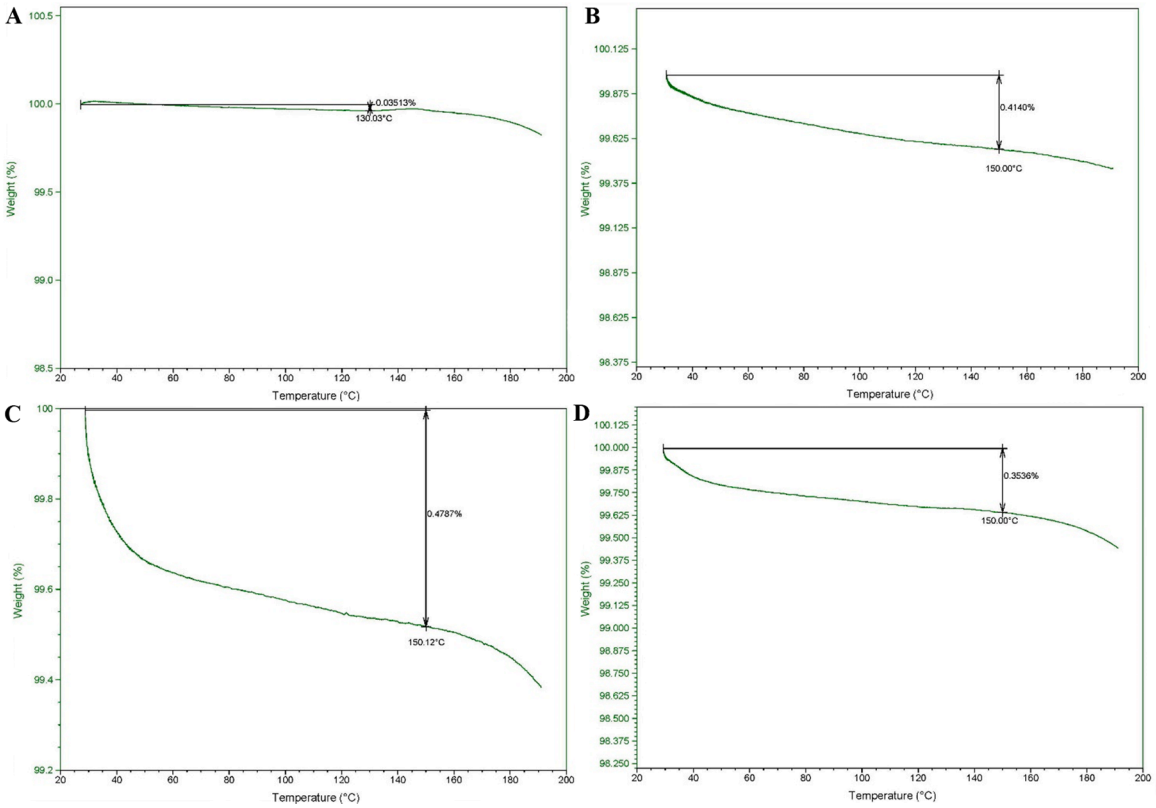


Fig. 2. TGA analysis of FM (A), Eudragit® E PO (B), physical mixture (C) and extrudate (D).

advancement is developing taste-masked FM.

Hot-melt extrusion (HME), which integrates fusion, mixing, shearing and compression in an axial space to blend materials at the molecular level, is a new pharmacological technique and plays an important role in the pharmaceutical industry (Vo et al., 2013). HME technology is widely used for masking taste, enhancing solubility (Alshehri et al., 2015),

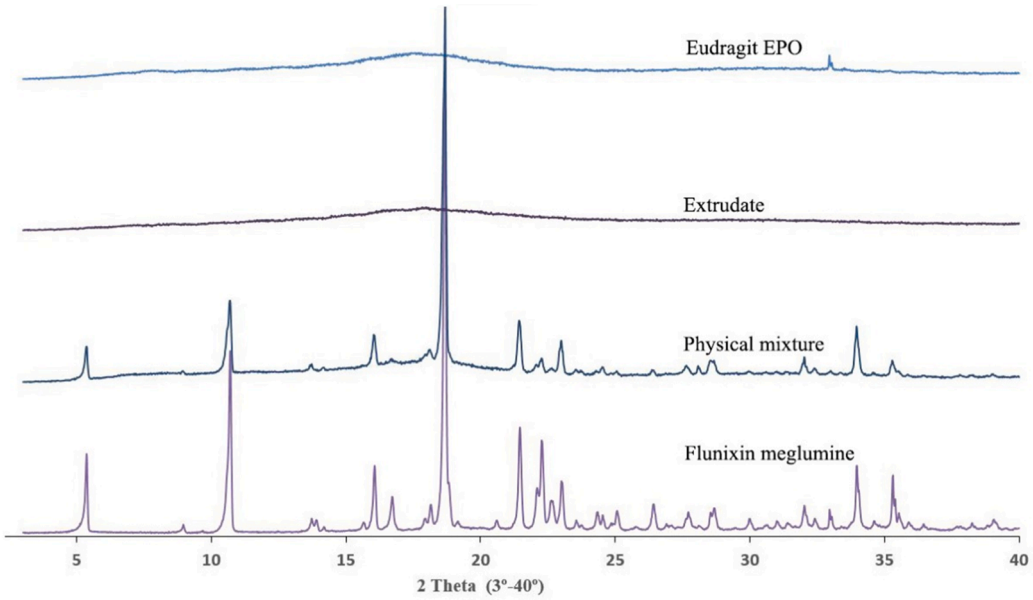


Fig. 3. PXRD analysis of FM, Eudragit® E PO, physical mixture and extrudate.

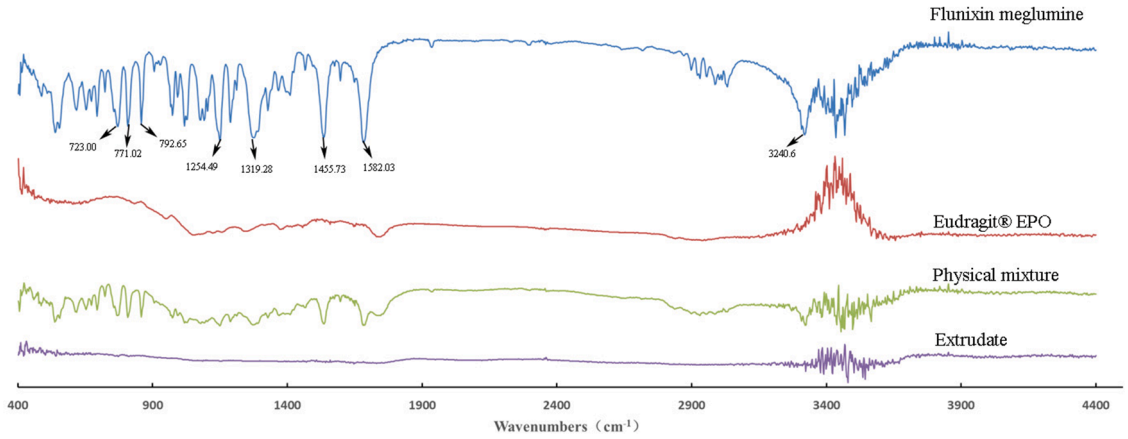


Fig. 4. FT-IR analysis of FM, Eudragit® E PO, physical mixture and extrudate.

achieving enteric release (Xu, 2018), preparing orodispersible formulations (Pimparade, 2017), etc. Furthermore, HME has advantages such as low cost, energy and time efficiency, and easy continuous operation (Ye et al., 2016).

Orally disintegrating tablets (ODTs) are solid formulations that contain medicinal substances and disintegrate rapidly in the oral cavity. Due to better patient compliance, ODT has attracted attention as a preferred alternative to conventional tablets and capsules. ODT could ingratiate enhanced life-cycle management, convenient dosing for patients with dysphagia in paediatrics, geriatrics and psychiatrics, and small animal treatments (Hirani et al., 2011; Takahiro and Tomohito; 2014, Yi, 2017).

In this study, for the first time, HME and ODT were used to prepare a taste-masked FM formulation. The formulation was systematically characterized, including content determinations, weight difference, friability, tensile strength, disintegration time, taste masking, stability, *in vitro* dissolution and *in vivo* pharmacokinetics, therefore providing a theoretical basis for use in veterinary clinics.

2. Materials and methods

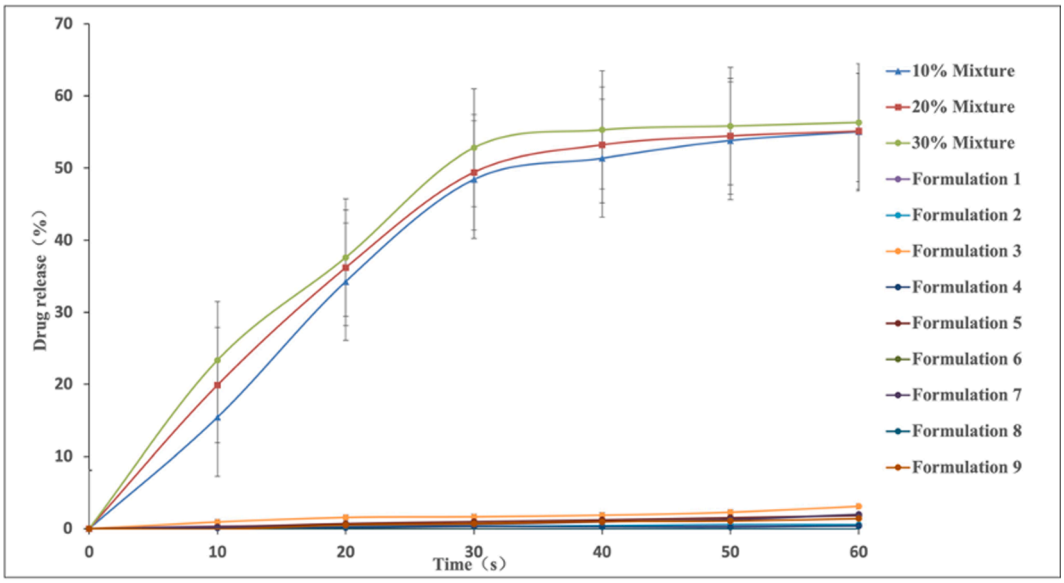
2.1. Materials

FM standard was purchased from the China Institute of Veterinary Drug Control (Beijing, China). FM was purchased from Qilu'shenghua Pharmaceutical Co., Ltd. (Dezhou, Shandong, China). Eudragit® E PO was obtained from Shenzhen Youpuhui Pharmaceutical Co., Ltd. (Shenzhen, Guangdong, China). Low-substituted hydroxypropyl cellulose (L-HPC), microcrystalline cellulose (MCC), mannitol (Man), magnesium stearate (MS) and other medicinal excipients were kindly gifted by Anhui Sunhere Pharmaceutical Excipients Co., Ltd. (Huainan, Anhui, China). All other chemical reagents used for HPLC analysis and dissolution were of analytical grade.

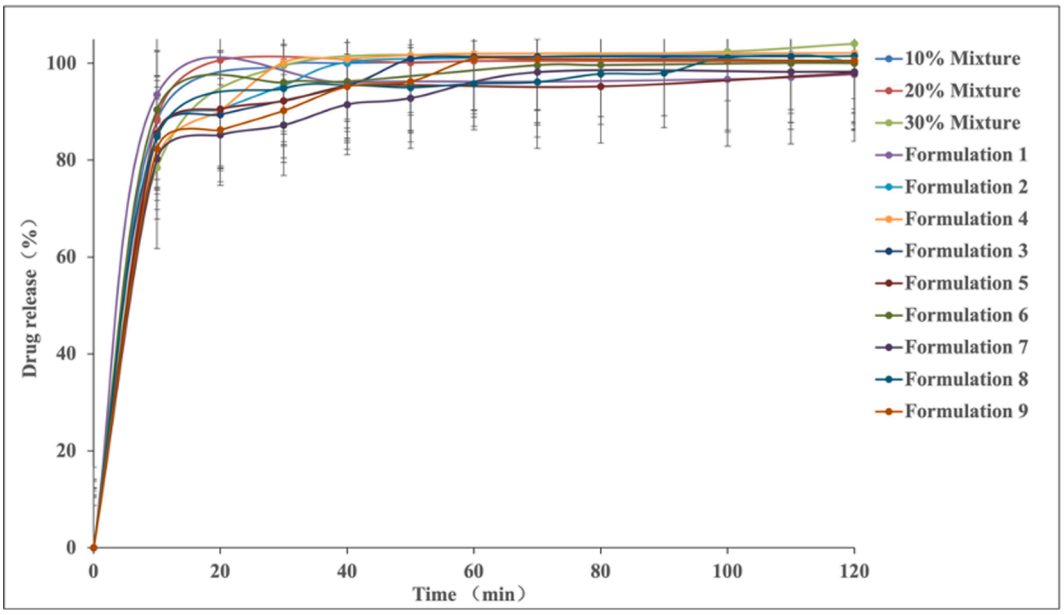
2.2. Methods

2.2.1. Preparation of the HME extrudate

Eudragit® E PO was chosen as the matrix. After passing through the



A



B

Fig. 5. Drug release of FM extrudate in artificial saliva (A) and in pH 4.5 buffer solution (B).

ASTM #30 mesh, FM and Eudragit® E PO were mixed using a V-shell blender (GlobePharma, Maxiblend®, New Brunswick, NJ, USA). A co-rotating twin-screw extruder (16 mm Prism Euro Lab, Thermo Fisher Scientific, Pittsburgh, PA, USA) was used to conduct HME. Then, FM-E PO extrudate was milled and sieved to a specific size (250~600 μm) and stored in glass vials. The HME parameters were optimized as follows: extrusion temperature, 135℃; screw speed, 100 rpm; and drug loading, 20%, which resulted in good process reproducibility.

2.2.2. Thermogravimetric analysis (TGA)

The thermal stability of the active pharmaceutical ingredient (API) and excipients was estimated by TGA (PerkinElmer Pyris 1, Shelton, CT, USA). The samples were heated from 30 to 200°C at a heating rate of 20°C/min under an inert nitrogen atmosphere at a flow rate of 20 ml/min. The results were analysed using Pyris software (PerkinElmer Life and Analytical Sciences, 719 Bridgeport Ave., CT, USA).

2.2.3. Differential scanning calorimetry (DSC)

A PerkinElmer Diamond differential scanning calorimeter equipped with Pyris software (Shelton, CT, USA) was used to conduct DSC analysis (Feng et al., 2015). Approximately 3~5 mg each of pure API, a physical mixture of FM and Eudragit® E PO and milled extrudate were hermetically sealed in aluminium pans. DSC parameters were set as heating from 30 to 200°C at 20°C/min in an inert nitrogen atmosphere with a flow rate of 20 ml/min.

2.2.4. Powder X-ray diffraction (PXRD)

The crystallinity of FM in HME extrudate was analysed by a powder X-ray diffraction apparatus (Bruker AXS, Madison, MI, USA). The machine was set at room temperature utilizing CuKα radiation at 15 mA and 30 kV, 4°/min and diffraction angles (2θ) of 1-40°.

2.2.5. Fourier transform infrared (FT-IR) spectroscopy

An Agilent Cary 660 FT-IR spectrometer (Agilent Technologies,

Table 3
Mixture design for composition optimization.

Run	A: MCC (%)	B: Man (%)	C: L-HPC (%)
1	18.750	52.500	3.750
2	41.250	31.125	2.625
3	30.000	41.250	3.750
4	26.250	45.000	3.750
5	41.250	30.000	3.750
6	31.125	42.375	1.500
7	18.750	52.500	3.750
8	41.250	32.250	1.500
9	25.781	47.156	2.062
10	21.000	52.500	1.500
11	21.000	52.500	1.500
12	41.250	32.250	1.500
13	41.250	30.000	3.750
14	41.250	31.125	2.625
15	34.500	39.000	1.500
16	35.096	35.906	3.187

Table 4
The results of the mixture design (pressure 4, mean ± SD, $n = 6$).

Run	Porosity (%)	Tensile strength (MPa)	Disintegration time (s)
1	0.667 ± 0.011	0.677 ± 0.018	18.1 ± 0.842
2	0.671 ± 0.120	0.965 ± 0.025	61.8 ± 1.28
3	0.693 ± 0.014	1.04 ± 0.010	18.5 ± 0.620
4	0.684 ± 0.023	0.974 ± 0.010	24.8 ± 1.30
5	0.650 ± 0.147	1.40 ± 0.013	31.9 ± 1.510
6	0.661 ± 0.032	0.958 ± 0.013	21.0 ± 1.932
7	0.67 ± 0.020	0.672 ± 0.023	19.5 ± 0.841
8	0.675 ± 0.011	1.24 ± 0.011	65.9 ± 2.56
9	0.668 ± 0.010	0.706 ± 0.027	18.2 ± 1.56
10	0.696 ± 0.020	0.578 ± 0.052	21.4 ± 0.944
11	0.700 ± 0.011	0.560 ± 0.020	22.6 ± 1.24
12	0.65 ± 0.020	1.250 ± 0.010	66.5 ± 1.62
13	0.650 ± 0.021	1.570 ± 0.030	32.5 ± 1.80
14	0.668 ± 0.040	0.950 ± 0.050	60.1 ± 0.51
15	0.671 ± 0.032	0.849 ± 0.031	38.2 ± 0.612
16	0.655 ± 0.022	0.969 ± 0.062	24.0 ± 0.751

Santa Clara, CA, USA) was used to assess the interactions between the API and excipient in the HME extrudate and in the physical mixture in the range of 400~4400 cm^{-1}.

2.2.6. Drug content tests

With an HPLC system comprising an e2695 separations module, a 2998 photodiode array detector, and a 717 plus autosampler (Waters Technologies Corporation, 34 Maple St., Milford, MA 0157, USA), chromatographic analysis was conducted at a wavelength of 282 nm utilizing a Phenomenex Luna C_{18} reversed-phase column (250 × 4.6 mm, 5 μm) (FLM Inc, Guangzhou, China). The mobile phase was composed of methanol and 0.1% potassium dihydrogen phosphate in a ratio of 80:20 (v:v) using a flow rate of 1.0 ml/min. All content determination data were calculated using flunixin (FX).

2.2.7. In vitro dissolution of the FM extrudate

According to the US Pharmacopeia (USP), *in vitro* two-stage drug release development was performed. Specifically, 150 ml of artificial saliva medium and 900 ml of buffer stage medium (pH 4.5) were used with a USP II apparatus.

The artificial saliva medium consisted of the following: $CaCl_2 \bullet 2H_2O$, 0.228 g; $MgCl_2 \bullet 6H_2O$, 0.061 g; NaCl, 1.017 g; $K_2CO_3 \bullet 1.5H_2O$, 0.603 g; $Na_2HPO_4 \bullet 7H_2O$, 0.204 g; and $NaH_2PO4 \bullet H_2O$, 0.273 g dissolved in 1000 ml of water and adjusted to pH 6.8 with 0.1 M HCl (0.826 ml HCl to 1000 ml of water). The buffer stage medium consisted of 2.99 g of $CH_3COONa \bullet 3H_2O$ dissolved in approximately 800 ml of water and then mixed with 14 ml of 2 mol/L HCl solution; then, water was added to increase the volume to 1000 ml.

Table 5
ANOVA results for the effect of the dependent variable.

Dependent variable	Source	Sum of squares	*df*	Mean square	*F*-value	*P*-valueProb > F
P: (%)	Model	2.694*E*-003	6	4.490*E*-004	6.92	0.0055
	Linear mixture	1.245*E*-003	2	6.223*E*-004	9.60	0.0059
	AB	8.214*E*-004	1	8.214*E*-004	12.67	0.0061
	AC	3.451*E*-006	1	3.451*E*-006	0.053	0.8227
	BC	3.629*E*-006	1	3.629*E*-006	0.056	0.8183
	ABC	1.424*E*-003	1	1.424*E*-003	21.95	0.0011
	Residual	5.836*E*-004	9	6.485*E*-005		
	Lack of Fit	5.836*E*-004	4	1.459*E*-004		
	Pure Error	0.000	5	0.000		
	Cor total	3.278*E*-003	15			
TS: (MPa)	Model	0.86	2	0.43	25.67	< 0.0001
	Linear mixture	0.86	2	0.43	25.67	< 0.0001
	Residual	0.22	13	0.017		
	Lack of Fit	0.22	8	0.027		
	Pure Error	0.000	5	0.000		
	Cor total	1.07	15			
DT: (s)	Model	5027.60	5	1005.52	41.33	< 0.0001
	Linear mixture	3519.06	2	1759.53	72.32	< 0.0001
	AC	6.81	1	6.81	0.28	0.6084
	BC	0.66	1	0.66	0.027	0.8726
	BC (B-C)	753.45	1	753.45	30.97	0.0002
	Residual	243.31	10	24.33		
	Lack of Fit	243.31	5	48.66		
	Pure Error	0.000	5	0.000		
	Cor total	5270.92	15			

A physical mixture and HME extrudate (equivalent to 20 mg of FX) samples were filled into hard shell hydroxypropyl methylcellulose (HPMC) capsules for the *in vitro* dissolution test. A Hanson SR9-plus™ dissolution apparatus (Chatsworth, CA, USA) was used for the two-phase release study at 100 rpm for 60 s (artificial saliva medium) and 120 min (pH 4.5, buffer stage medium) at 37°C (n = 3). Aliquots (2 ml) were withdrawn every 5 s for 60 s and then every 10 min for 2 h, and 2 ml of fresh medium was added to maintain the sink condition after each sampling. A Waters HPLC system was used to analyse the samples. The dissolution parameters were also used for testing ODT.

2.2.8. Optimization of the formulation and preparation of taste-masked FM ODT

The compressibility, disintegration, solubility and powder of the excipients were investigated. The ingredients with the best properties were selected, and their dosage ranges were confirmed. Then, the D-optimal mixture design was used to filter the best composition by tensile strength, disintegration time and porosity.

The optimized composition was used for the final formulation of ODT. The milled and sieved FM SD was mixed with other excipients in a V-shell blender for 20 min at 20 rpm. In the last 2 min, magnesium stearate was added. A 10 mm standard flat-faced punch was used for direct compression. The pressure was adjusted to maintain an

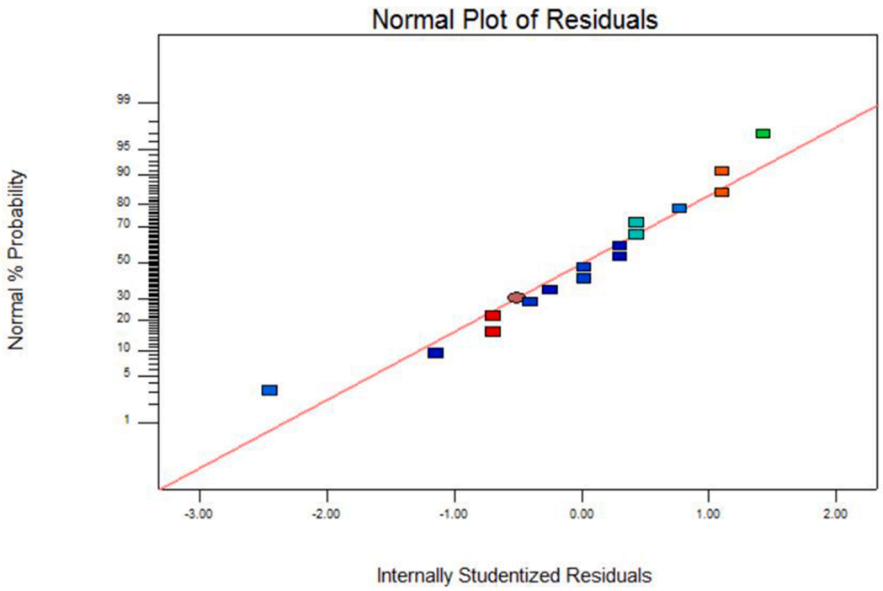


Fig. 6. The normal plot of the residuals.

appropriate tensile strength.

2.2.9. Tablet properties

Weight variations were measured on a microbalance. Briefly, twenty tablets were weighed, and the average weight was determined. Each tablet weight was compared to the average weight and evaluated within the USP specified tolerances for uncoated tablets (± 7.5%).

Tablet friability was assessed by a fabricator tester (EF-1 W, Electrolab, Mumbai, India). It rotated for 4 min at 25 rpm continuously. Before the test, the tablets were weighed accurately and then dusted and reweighed carefully after the test.

Hardness was measured by a hardness tester (TS-50N, Okada Seiko Co., Ltd., Japan). The tensile strength (TS) was calculated by the following formula: $TS = 2F/\pi DH$, where F is the crushing load, D is the diameter and H represents the thickness.

Porosity was calculated by the formula $\varepsilon = 1 - m/(\rho_t V)$, where ρ_t is the true density, m is the weight, and V is the volume.

Disintegration time was tested by a pharmacopoeia apparatus (Erweka ED-2 L, Heusentamm, Germany). The disintegration medium was distilled water.

2.2.10. Evaluation of taste-masked effectiveness

E-tongue evaluation. Measurements were conducted at 37°C using an electric tongue (TS-5000Z, INSENT, Japan) (Table 1 Samples for E-tongue analyses). Test solutions included reference solution (artificial saliva): KCl + tartaric acid; negative electrode cleaning solution: distilled water + ethanol + HCl; and positive electrode cleaning solution: KCl + distilled water + ethanol + KOH. The sensors used were C00, AE1, AN1 and BT0, corresponding to the tastes of acidic bitterness, astringency, basic bitterness and hydrochloride bitterness, respectively. All calculations and analyses were performed by system-provided software.

E-nose evaluation. The E-nose system (PEN3, AIRSENSE, Germany) contains 10 different metal-oxide sensors. Samples were the same as E-tongue analysis except the sample weight was 2 g. The test conditions were set as: test time, 1 s/group; cleaning time, 100 s; sensor zero-time, 5 s; sample setup time, 5 s; flow rate, 400 ml/min; record time, 100 s; and cleaning flow rate, 400 ml/min.

In vitro dissolution of ODT. A two-stage drug-release study *in vitro* was conducted. Then, 150 ml artificial saliva medium and 900 ml buffer solution (pH 4.5) were used with a USP II apparatus. The specific parameters are described in Section 2.2.7.

2.2.11. Physical and chemical stability

The physical and chemical stability of FM extrudate were tested by storage in closed glass vials under 25°C/65% RH storage conditions for 12 months and 40°C/75% RH accelerated conditions for 6 months. The physical stability was evaluated by DSC, and drug content analysis was used to investigate the chemical stability. The same conditions were used for the stability test of ODT. The dissolution similarity factor (f_2) was utilized to compare the dissolution profiles of ODT following the stability tests.

2.2.12. Pharmacokinetic study

Grouping and drug administration. A comparative pharmacokinetic study of FM ODT (TEST), commercially available FM injection (XINNIKA®) and FM granules (HAIYANSHU®) was performed in beagle dogs at the National Beijing Center for Drug Safety Evaluation and Research.

Six beagle dogs weighing approximately 10 kg were obtained from a local company (Beijing Rixin Technology Co., Ltd., No. 111006700000135). The dogs were housed separately, fed a commercial dry diet twice a day and water *ad libitum*. The protocol was reviewed and approved by the National Beijing Center for Drug Safety Evaluation and Research Experimental Animal Welfare and Ethics Committee (protocol number IACUC-2017-081).

A randomized 3 × 3 crossover design experiment was conducted. TEST and HAIYANSHU® were given orally (p.o.) at a dose rate of 2 mg/kg (calculated based on FX). XINNIKA® was administered intravenously (i.v.) via the radial veins at a dose of 2 mg/kg utilizing a 2-ml disposable syringe with a #6 needle. The washout period was two weeks.

Blood sample collection. Blood samples were collected before (0 min) injection and 5 min, 10 min, 15 min, 30 min, 45 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 9 h, 12 h, 16 h, 24 h, 36 h, 48 h and 72 h after injection. For others, samples were collected before (0 min) administration and 5 min, 15 min, 30 min, 45 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 9 h, 12 h, 16 h, 24 h, 36 h, 48 h and 72 h after administration. Samples were centrifuged at 3000 × g for 10 min. The plasma was divided into 2 aliquots, transferred

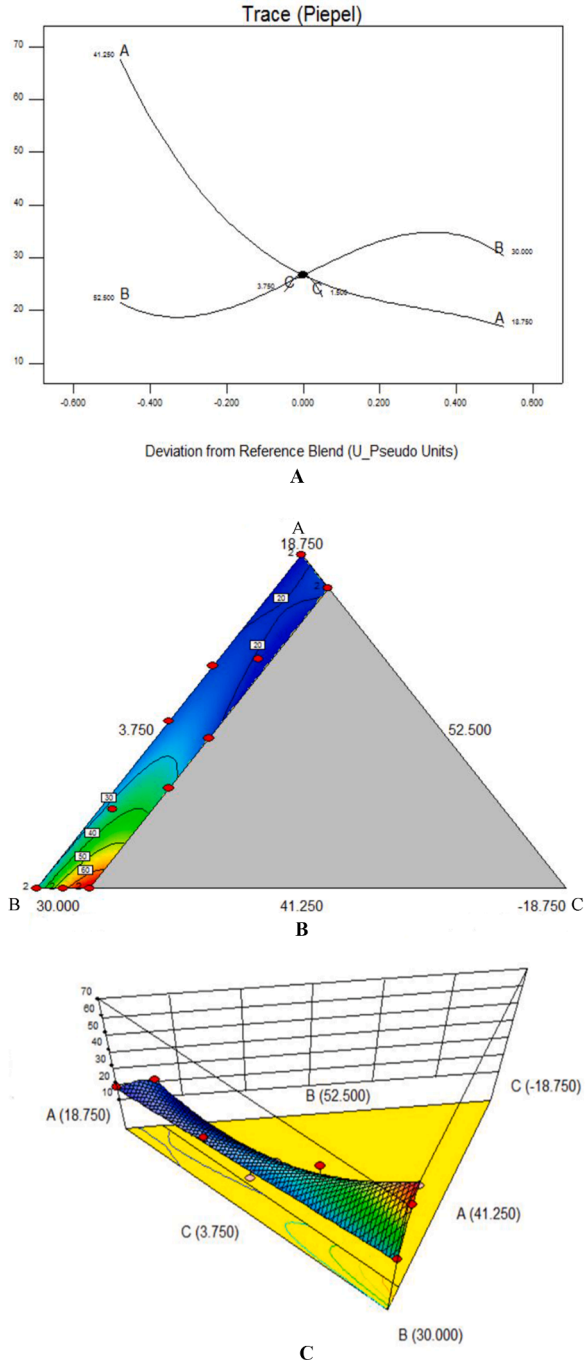


Fig. 7. D-optimal function results: curve (A); contour line (B); 3D fractal image (C).

Table 6
Composition optimization results of the D-optimal mixture design.

Number	MCC (%)	Man (%)	L-HPC (%)	TS (%)	DT (%)
1	18.750	52.500	3.750	0.696146	16.9525
2	28.465	45.000	1.535	0.778911	18.0344
3	25.887	47.350	1.764	0.729647	17.7072
4	20.156	51.094	3.750	0.732489	17.7837
5	19.610	51.640	3.750	0.718359	17.4605
6	20.561	50.689	3.750	0.742945	18.0228
7	24.374	48.810	1.816	0.694505	17.9689
8	27.222	46.137	1.641	0.75483	17.6132

Table 7
Composition verification of the D-optimal mixture design (mean ± SD, $n = 3$).

Property	20170308	20170315	20170322
Friability (%)	0.88 ± 0.08	0.87 ± 0.06	0.82 ± 0.02
Tensile strength (MPa)	0.72 ± 0.0074	0.714 ± 0.010	0.727 ± 0.042
Disintegration (s)	17.52 ± 1.44	16.58 ± 1.73	18.13 ± 1.02
Weight variation (%)	2.72 ±1 .02	3.13 ± 1.12	3.13 ± 1.14%

to cryovials and stored at -20°C.

Drug assay. Extraction of FM from blood samples and validation of an HPLC method for the analysis of FM in plasma were performed in accordance with methods described elsewhere (Huber et al., 2013), with a few modifications. Briefly, 400 μl of plasma was transferred to a 10 ml tube. Then, 40 μl of HCl (1 mol/L) and 4 ml of acetonitrile were added with mixing for 30 s after the addition of each component, and the samples were subsequently centrifuged at 10,000 rpm for 10 min. The organic phase was transferred to another tube, and acetonitrile and HCl were added to the rest for another extraction. The combined organic phase was dried under N_2 at 45°C, redissolved in 400 μl mobile phase and filtered through a 0.22 μm organic filter for HPLC analysis.

2.2.12.4. Statistics. Microsoft Office Excel 2019 was used to calculate all pharmacokinetic parameters according to the noncompartment model method. The results are expressed as the means ± standard deviation (SD). Differences between FM ODT and granules were considered to be significant at $P \leq 0.05$ by t-test or via the rank-sum test for parameters that were not normally distributed.

3. Results and discussion

3.1. Hot-melt extrusion process

3.1.1. Selection of matrix

The compatibility of the matrix and API, thermal stability, and glass-transition temperature (T_g) were measured before HME. Compatibility can be assessed based on the solubility parameters of each component. The solubility parameter can be calculated by the Hoftyzer and Van Krevelen methods using the following equation: $\delta t^2 = \delta d^2 + \delta p^2 + \delta h^2$, where δd^2, δp^2, and δh^2 denote the dispersion, polarity, and hydrogen bond solubility parameters, respectively (Hansen, 2000). The solubility parameter of FM was calculated as 34.29 $MPa^{0.5}$ and Eudragit® E PO is 39.105 $MPa^{0.5}$. It is generally accepted that a D-value less than 7 $MPa^{0.5}$ between the API and the excipients indicates good compatibility (Mohammad et al., 2011). Eudragit® E PO is an amorphous cationic copolymer formed by dimethyl amino ethyl methacrylate and neutral methacrylic acid ester and is widely used due to its masked taste, controlled release, and solubility, among other advantages (Li et al., 2015). The interaction between Eudragit® E PO and FM was confirmed by FT-IR. These interactions may have aided in improving the taste-masked effect and drug release.

T_g should be close to prevent poor stability of the extrudate or API degradation at high temperature. All components did not show degradation during the TGA test heating to 150 ℃. Eudragit® E PO has good thermal stability and a low glass-transition temperature (Kojima et al., 2012). FM has a melting point of approximately 140℃; however, the physical mixture could be extruded at a low temperature (135℃) without extra plasticizer, which indicates that FM may act as a plasticizer during HME, suggesting that Eudragit® E PO is suitable for this application.

3.1.2. Parameter optimization for HME

The effects of drug loading, extrusion temperature and screw speed were conducted by single-factor tests, and an orthogonal test [$L_9(3^4)$] was conducted for optimization. Orthogonal test results were evaluated

Table 8
Results of E-tongue analyses (mean ± SD, $n = 3$).

Samples	B-bitterness2 AN0	Aftertaste-B C00	Aftertaste-A AE1	H-bitterness BT0	Bitterness C00	Astringency AE1
P6.8	0	0	0	0	0	0
FM	4.27 ± 0.26	4.32 ± 0.34	10 ± 0.72	0.14 ± 0.01	23.89 ± 0.38	17.8 ± 0.16
PM-FM	1.87 ± 0.36	4.88 ± 0.38	17.79 ± 0.83	0.45 ± 0.02	25.64 ± 0.24	25.53 ± 0.12
PM-Placebo	4.59 ± 0.55	0.1 ± 0.03	1.18 ± 0.27	-0.01 ± 0	2.34 ± 0.27	2.61 ± 0.39
H-FM	1.52 ± 0.18	1.36 ± 0.07	3.12 ± 0.32	0.06 ± 0.01	16.48 ± 0.27	6.96 ± 0.25
H-Placebo	4.37 ± 0.35	0.03 ± 0.03	0.39 ± 0.06	0 .00± 0.01	0.82 ± 0.14	0.46 ± 0.04
G-FM	5.39 ± 0.41	5.58 ± 0.43	14.98 ± 0.66	0.19 ± 0.06	26.71 ± 0.45	23.00 ± 0.27
O-FM	5.28 ± 0.27	3.81 ± 0.2	10.39 ± 0.49	0.09 ± 0.01	23.45 ± 0.31	17.84 ± 0.22
O-H-FM	3.71 ± 0.19	0.51 ± 0.03	1.25 ± 0.16	-0.02 ± 0.02	9.06 ± 0.24	2.58 ± 0.23
O-Placebo	2.78 ± 0.21	0.14 ± 0.02	0.48 ± 0.08	-0.01 ± 0.03	2.07 ± 0.19	0.7 ± 0.04

Note: P6.8 indicates artificial saliva.

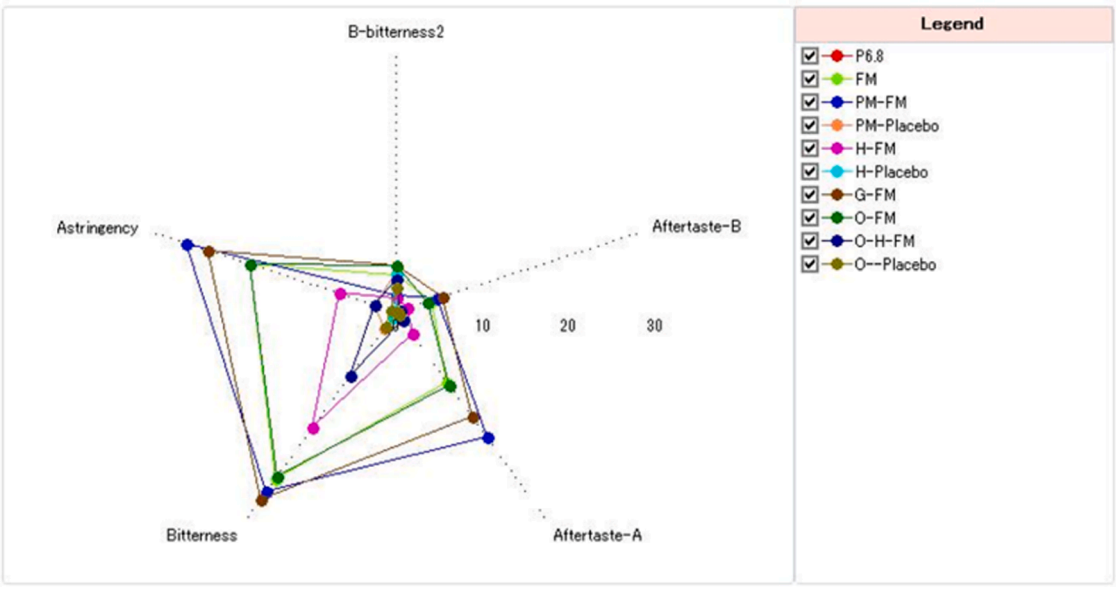


Fig. 8. Radar chart of the effective index.

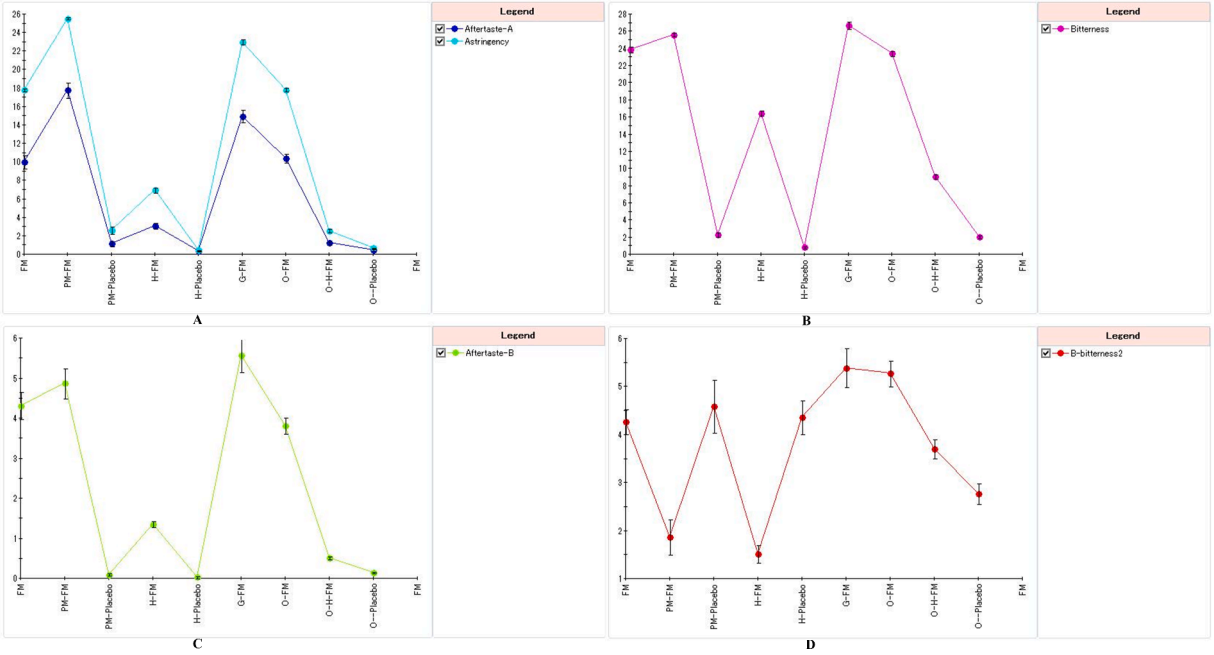


Fig. 9. Aftertaste-A and astringency (A), bitterness (B), aftertaste-B (C), and B-bitterness2 (D).

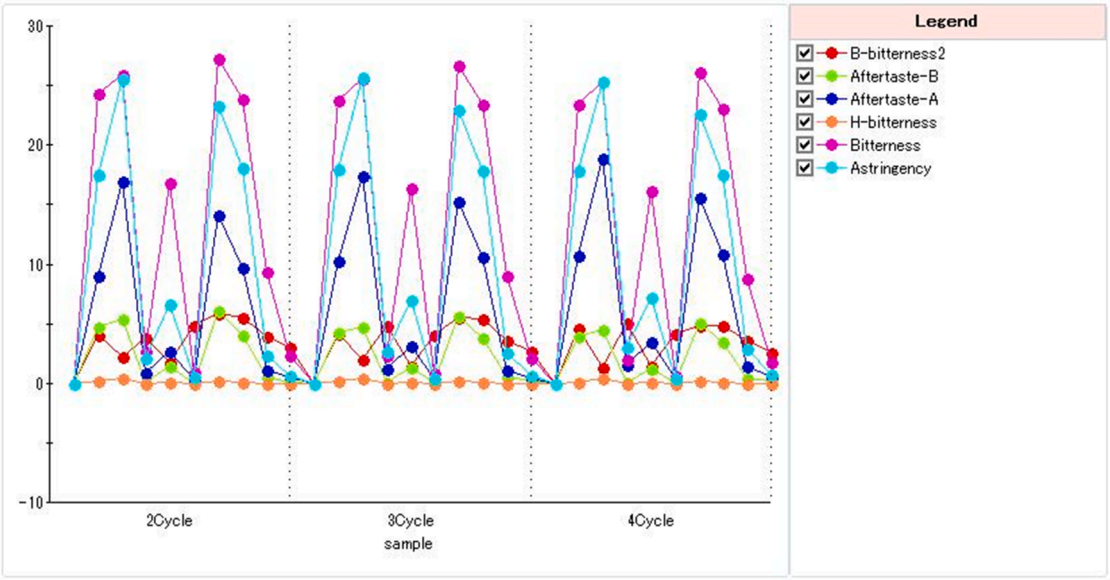


Fig. 10. Reproducibility of the E-tongue analysis.

by artificial saliva medium dissolution tests. Variance analysis (Table 2) showed that the different levels did not cause significant differences in dissolution. Thus, considering the clinical demands and production costs, HME parameters were optimized as follows: extrusion temperature, 135℃; screw speed, 100 rpm; drug loading, 20%; and the process reproducibility was good.

Extrusion temperature and screw speed could affect HME results: Sarode AL reported that the physicochemical properties of HPMCAS were not significantly affected by HME, but the most significant change was the release of acetic and succinic acids with increasing HME temperature and speed, which in turn affected the dissolution time (Sarode et al., 2014). To achieve a high dissolution speed and supersaturation of poorly water-soluble drugs, the extrusion temperature and screw speed should be well controlled to improve the drug-polymer interactions (Fukuda et al., 2013). This study preliminarily optimized the HME parameters, and further optimization should be conducted in the scale-up experiment.

3.1.3. Characterization and in vitro study of HME extrudate

Differential scanning calorimetry (DSC). FM showed an endothermic peak at 141.03°C. HME extrudate and Eudragit® E PO showed no peak, while the physical mixture exhibited a less intense endothermic peak than FM at the same temperature (141.03°C), which proved that FM was completely converted into an amorphous state during HME (Fig. 1).

Thermogravimetric analysis (TGA). TGA thermograms (Fig. 2) proved the thermal stability of FM, Eudragit® E PO, physical mixture, and HME extrudate. No sample showed significant degradation (< 1%) up to 150°C. This proved the thermal stability during HME and confirmed the feasibility.

Powder X-ray diffraction (PXRD). The characteristic peaks in PXRD (Fig. 3) of crystalline FM were shown at 2θ values of 5.35°, 10.70°, 16.08°, 18.68°, 21.44°, 23.01°, 33.98° and 35.31°, and these peaks were observed in the spectrogram of the physical mixture, while HME exudate and Eudragit® E PO exhibited no peak, confirming the crystalline state change during HME.

Fourier transform infrared (FT-IR) spectroscopy. FT-IR results (Fig. 4) showed characteristic peaks at 723.00, 771.02, 792.02, 1254.49, 1319.28, 1455.73, 1506.15 and 3316.9 cm^{-1} in the spectra of FM and the physical mixture, while no prominent peak was present in the spectra of Eudragit® E PO and HME extrudate.

In the FT-IR spectrum, the shift, decreased intensity and absence of characteristic peaks might be due to intermolecular interactions, which indicated the generation of hydrogen bonds during the extrudate.

It was reported that FT-IR spectra of HME-based solid solutions of artesunate in the water-soluble polymers Soluplus® and Kollidon® VA64 did not exhibit any changes in the molecular stretching bands (although slight shifts in specific peaks were observed), but the dissolution properties of artesunate were significantly improved in the resulting molecular dispersions (Fule et al., 2015). Therefore, it is necessary to combine several characterizations. All the characterization results sufficiently confirmed that FM was converted from a crystalline to an amorphous state through HME with Eudragit® E PO.

In vitro dissolution studies and drug content test. *In vitro* dissolution results (Fig. 5) showed FM extrudate and physical mixture had dissolutions of <4% and >50%, respectively, at 1 min in artificial saliva medium and >80% dissolution at 2 h in pH 4.5 buffer solution. The drug content test indicated that there was no API lost during HME (HPLC data not shown).

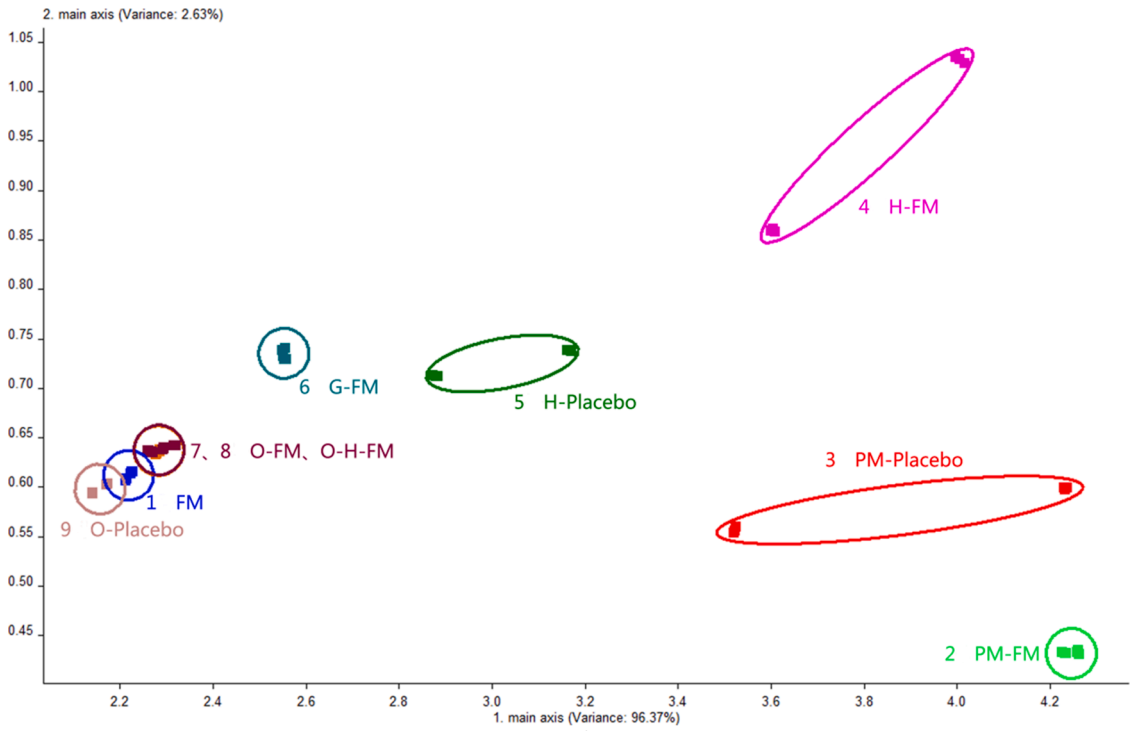
In brief, a taste-masked FM solid dispersion was successfully formulated, and it maintained the quick release feature.

3.2. Optimization of the formulation and preparation of ODT

The content ranges of MCC (A), man (B) and L-HPC (C) were set as 20~50%, 45~75%, and 2~5%, respectively. The MS content was tested, but different levels caused no significant variations, and the recommended amount of 1% was chosen.

Based on preliminary studies, a D-optimal mixture design was conducted. Five compression forces were tested to determine the optimal force (16 formulations were tested for each force, Table 3). The fourth force (Table 4) was chosen because it achieved a proper hardness of approximately 2 kgf (Kuno et al., 2005). The statistical significance was analysed by regression fitting of a linear model, a two-term multinomial model and an incomplete three-term model using Design Expert 11.

The fitting equations of porosity (y_1), tensile strength (y_2) and disintegration time (y_3) are as follows (ratio of MCC, x_1; ratio of Man, x_2; ratio of L-HPC, x_3):



A

PCA-Analysis

normalization :	PCA :
Matrix :	Correlation-M.
Algorithm:	PCA
Variance: :	99.003 %
1. main axis:	96.372 %
2. main axis:	2.6315 %

Discrimination power:

	1	2	3	4	5	6	7	8	9
1		1.000	0.917	0.968	0.941	0.999	0.978	0.989	1.000
2	1.000		0.451	0.977	0.977	1.000	1.000	1.000	1.000
3	0.917	0.451		0.942	0.732	0.884	0.910	0.896	0.921
4	0.968	0.977	0.942		0.837	0.952	0.964	0.958	0.969
5	0.941	0.977	0.732	0.837		0.856	0.934	0.921	0.949
6	0.999	1.000	0.884	0.952	0.856		0.995	0.988	0.997
7	0.978	1.000	0.910	0.964	0.934	0.995		0.577	0.979
8	0.989	1.000	0.896	0.958	0.921	0.988	0.577		0.921
9	1.000	1.000	0.921	0.969	0.949	0.997	0.979	0.921	

B

Fig. 11. Principal component analysis (A) and distinction analysis of PCA (B).

$$y_1 = 0.02x_1 + 0.016x_2 + 0.46x_3 - 5.08 \times 10^{-4}x_1x_2 - 0.011x_1x_2 - 8.80 \times 10^{-3}x_2x_3 + 1.88 \times 10^{-4}x_1x_2x_3$$

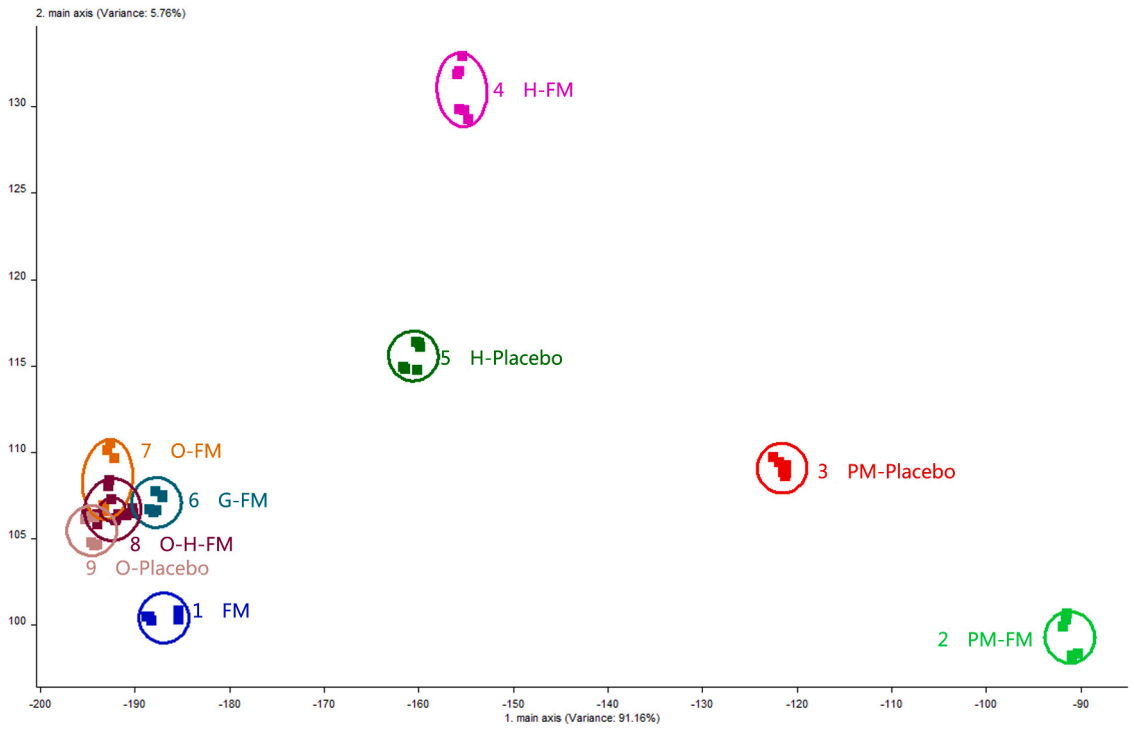
$$y_2 = 0.025x_1 - 9.78 \times 10^{-4}x_2 + 0.075x_3$$

$$y_3 = 7.24x_1 + 1.74x_2 + 66153.92x_3 - 0.35x_1x_2 - 1391.73x_1x_2 - 1351.89x_2x_3 + 13.18x_1x_2x_3 - 1.99 \times 10^{-3}x_1x_3(x_1 - x_3) + 6.26x_2x_3(x_2 - x_3)$$

The fitting models all had high degrees of significance for the effect of the dependent variable according to the ANOVA results (Table 5): porosity, F = 6.92, $P<0.01$; tensile strength, F = 25.67, $P<0.0001$; and disintegrating time (DT), F = 41.33, $P<0.0001$. These results indicated a good correlation between the predicted values from the models and the experimental values.

As disintegration time (DT) is the most important index for ODTs, the optimization was based on the DT. By using the optimization function, the software predicted random combinations until the optimal target response value was achieved. The residuals conform to a normal distribution, indicating a good fit (Fig. 6). The trend of the influence on the disintegration time was represented as a parabola with a global minimum and arms with large tangent values (Fig. 7A), and the contour map was a semiellipse, which showed that the factors had different interaction degrees (Fig. 7B). The 3D fractal image of the DT showed the effects of different combinations (Fig. 7C). All of these results indicated a good fit of the polynomial regression model of DT, and the predicted solutions were reliable.

Several solutions were predicted, and the tensile strength and disintegration time both met the requirements (Table 6). According to a previous study, the mixture with a higher proportion of Man was chosen



A

LDA-Analysis

normalization :	LDA :
Matrix :	Correlation-M.
Algorithm:	LDA
Variance: :	96.914 %
1. main axis:	91.158 %
2. main axis:	5.7567 %

B

Fig. 12. Linear discriminant analysis (A) and contribution rate of LDA (B).

as the optimal composition: 25% FM milled extrudate, 18.75% MCC, 52.5% Man, 3.75% L-HPC and 1% MS.

For ODTs, it is necessary to choose proper components to achieve a reasonable tensile strength for packing and transport and a suitable porosity for quick water absorption to allow disintegration, but these two properties are contradictory (Gryczke et al., 2011; Al-Khattawi et al., 2014). Excipients should have good compression properties to ensure formability and disintegration. Therefore, appropriate pharmaceutical adjuvants should be reasonably matched and optimized.

MCC is primarily used as a binder/diluent in oral tablet and capsule formulations (Rowe, 2009). Man is widely used as a diluent in rapidly dispersing oral preparations (Lee et al., 2003). In addition, it is cheaper for veterinary use and has a shorter disintegrating time than xylitol and better fluidity than lactose. L-HPC is commonly used in the preparation of rapidly disintegrating tablets produced by direct compression methods (Douroumis et al., 2011; Yan et al., 2010).

Design experts are widely used in the design of experiments (DOE). It calculates the D-optimal design points in the experimental domain for the proposed model based on the candidate runs. This design enabled the evaluation of the appropriate regression model. Stepwise regression was performed on the special cubic model, where the interaction coefficients with the largest *P*-values were sequentially deleted until only significant interaction coefficients (*P*-value <0.05) remained in the model. The significant model was used to fit the responses. The lack-of-fit test and a normal probability plot of the residuals were used to evaluate the model and to detect outliers. Contour plots from the significant model of the responses were drawn to determine the optimal variable settings (Rambali et al., 2003). In this study, the optimal composition was determined by a D-optimal mixture design. The verification proved that the composition was suitably optimized.

3.3. Tablet properties

3.3.1. Composition verification

The tablet composition was verified (Table 7). Three batches of taste-masked FM ODT were manufactured, and the tablet properties were determined. The pharmacopoeia specifications of less than 1% friability, proper tensile strength, less than 30 s disintegration and less than 5% weight variation were all met.

3.3.2. Evaluation of taste-masking effectiveness

E-tongue test result is shown in Table 8. The radar chart (Fig. 8)

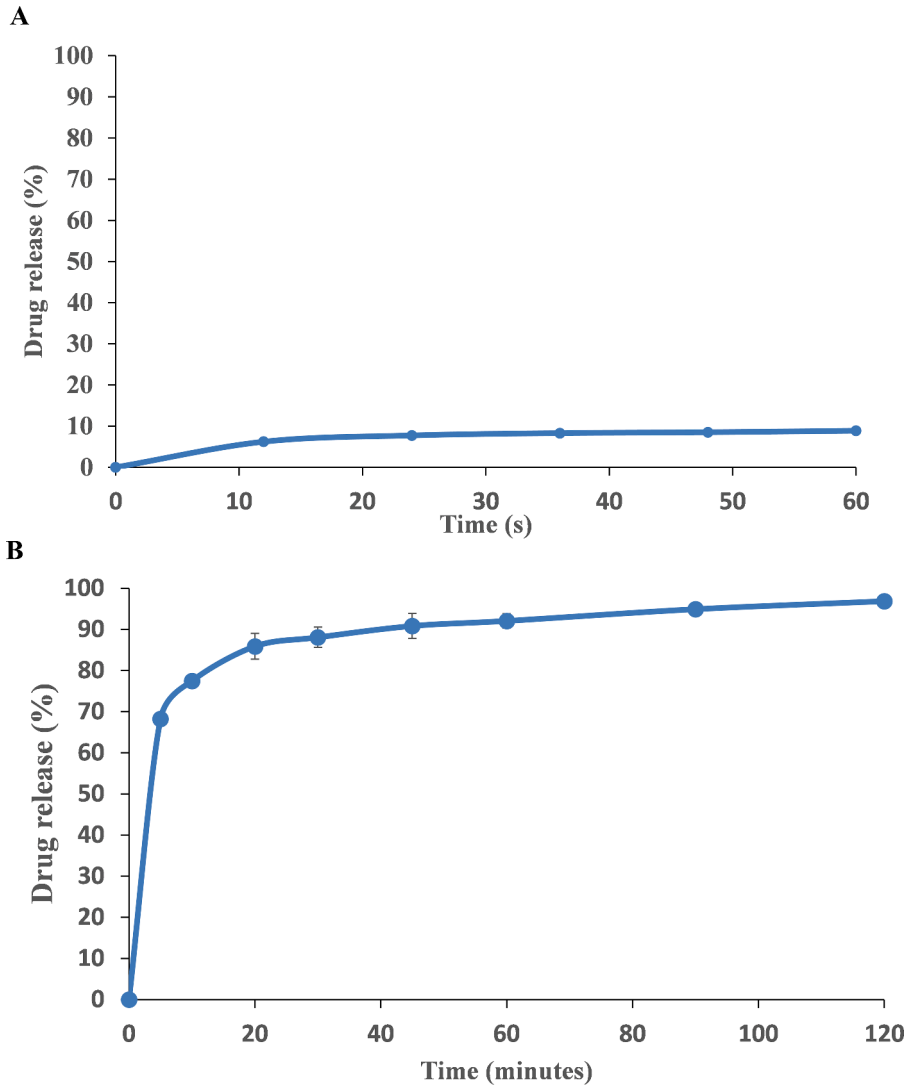


Fig. 13. Drug release of ODT in artificial saliva (A) and in pH 4.5 buffer solution (B).

Table 9
Drug content analysis of FM SD and FM masked ODTs (mean ± SD, $n = 3$).

FM SD (content, %) 40°C/75% RH		25°C/60% RH		FM ODT (labelled amount, %) 40°C/75% RH		25°C/60% RH	
0 m	19.91 ± 0.009	0 m	19.75 ± 0.027	0 m	99.27 ± 0.306	0 m	99.4 ± 0.346
1 m	19.90 ± 0.101	3 m	19.93 ± 0.069	1 m	99.33 ± 1.007	3 m	99.07 ± 2.27
2 m	19.94 ± 0.085	6 m	19.96 ± 0.082	2 m	99.13 ± 0.355	6 m	98.87 ± 0.503
3 m	19.77 ± 0.127	9 m	19.81 ± 0.092	3 m	98.80 ± 1.311	9 m	98.53 ± 0.902
6 m	19.88 ± 0.033	12 m	19.84 ± 0.093	6 m	99.33 ± 1.617	12 m	99.06 ± 0.636

showed that 9 samples had obviously different bitterness, aftertaste-B, astringency, aftertaste-A and B-bitterness2 values. O-H-FM had remarkably lower values than FM and G-FM in each index (Fig. 9). The reproducibility results showed that the E-tongue analysis was robust and reliable (Fig. 10).

E-nose response spectrograms showed that 9 samples had similar smells but differed in strength and ratio. Principal component analysis (PCA) and linear discriminant analysis (LDA) (Figs. 11 and 12) were conducted based on these spectrograms (data not shown). PCA results

Table 10
Plasma concentration of FM (μg/mL, mean ± SD, $n = 6$).

Time (h)	XINNIKA®		
0	—	—	—
0.083	45.74 ± 4.30	0.92 ± 0.45	0.31 ± 0.10
0.167	40.82 ± 5.43	—	—
0.25	32.10 ± 4.28	3.60 ± 0.82	1.36 ± 0.41
0.5	23.49 ± 4.42	6.45 ± 1.47	3.23 ± 0.29
0.75	14.88 ± 4.36	10.86 ± 2.78	7.08 ± 0.63
1	8.62 ± 1.27	9.00 ± 1.16	9.42 ± 1.22
1.5	6.48 ± 0.41	1.06 ± 2.31	7.50 ± 0.90
2	3.75 ± 0.63	4.93 ± 1.38	6.33 ± 1.15
3	2.59 ± 0.93	3.49 ± 0.76	4.17 ± 0.58
4	1.80 ± 0.53	2.41 ± 0.98	3.03 ± 0.61
6	1.41 ± 0.56	1.81 ± 0.32	2.10 ± 0.61
9	0.90 ± 0.35	1.26 ± 0.19	1.05 ± 0.56
12	0.54 ± 0.19	0.73 ± 0.25	0.76 ± 0.33
16	0.19 ± 0.11	0.49 ± 0.19	0.47 ± 0.17
24	—	0.14 ± 0.05	0.15 ± 0.08
36	—	—	—

Note: ND means the drug was not detectable.

revealed that the first main component accounted for 96.37% of the variance. According to the distinction analysis of PCA, the E-nose evaluation could remarkably distinguish the nine samples. LDA

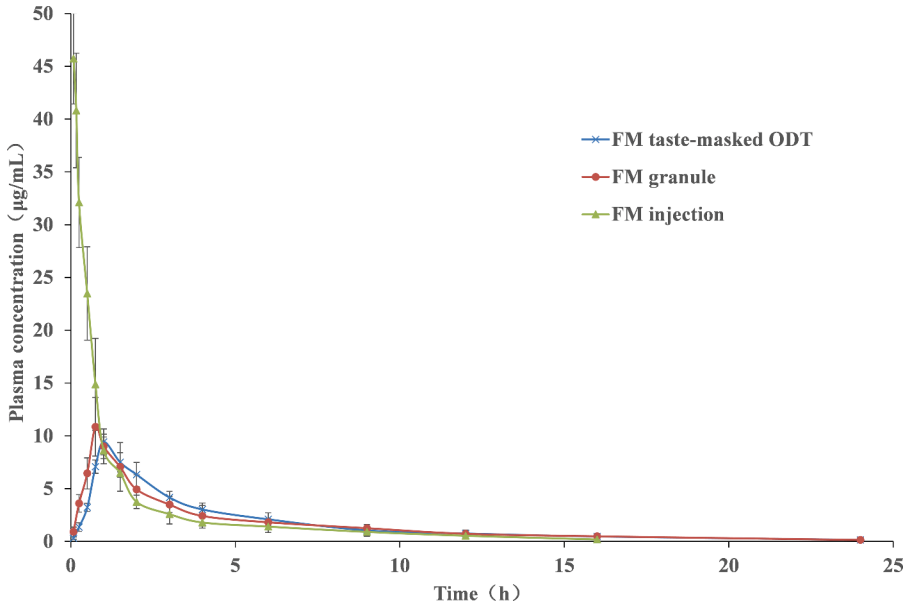


Fig. 14. The plasma concentration of FM following a single i.v. administration of commercial injection (XINNIKA®) and p.o. administration of taste-masked ODT and commercial granules (HAIYANSHU®) at a dose of 2 mg/kg body mass (Mean ± SD, $n = 6$).

Table 11
Pharmacokinetic parameters after a single i.v. injection of FM and p.o. administration of commercial FM granules and taste-masked FM ODT in dogs (mean ± SD, $n = 6$).

Parameter	XINNIKA®	TEST		HAIYANSHU®	
λ_z (1/h)	0.1934 ± 0.0450	0.1401 ± 0.0278		0.1576 ± 0.0349	
$t_{1/2\lambda}$ (h)	3.6822 ± 0.9511	5.1024 ± 0.9494		4.6049 ± 1.1588	
$AUC_{0-\infty}$ (μg•h/ml)	46.1597 ± 7.2448	39.2863 ± 6.5049		37.8925 ± 3.7929	
$AUMC_{0-\infty}$ (μg•h^2/ml)	135.5026 ± 36.1647	239.6223 ± 70.3842		219.1822 ± 42.9413	
$MRT_{0-\infty}$ (h)	2.9216 ± 0.4724	6.0127 ± 0.8541		5.8195 ± 1.2398	
MAT (h)	—	3.0911 ± 0.5089		2.8979 ± 0.8286	
C_{max} (μg/ml)	—	9.5283 ± 1.0684		11.04 ± 2.5642	
CL (L/h•kg)	0.0442 ± 0.0070	—		—	
$Vd_{(ss)}$ (L/kg)	0.1290 ± 0.0292	—		—	
F (%)	—	86.23 ± 16.26		83.37 ± 11.91	
		Median	Range	Median	Range
T_{max} (h)	—	1.00	1.00~1.50	0.75	0.75~1.00*

Note: Compared with commercial granules, * $P < 0.05$, ** $P < 0.01$.

Table 12
ANOVA of the pharmacokinetic parameters ($n = 6$).

Parameter	Test		HAIYANSHU®		*P*-value
	Mean	SD	Mean	SD	
λ_z (1/h)	0.1401	0.0278	0.1185	0.0334	0.251
$t_{1/2\lambda}$ (h)	5.1024	0.9494	4.6049	1.1588	0.435
$AUC_{0-\infty}$ (μg•h/mL)	39.2863	6.5049	37.8925	3.7929	0.660
$AUMC_{0-\infty}$ (μg•h^2/mL)	239.6223	70.3842	219.1822	42.9413	0.557
$MRT_{0-\infty}$ (h)	6.0127	0.8541	5.8195	1.2398	0.997
C_{max} (μg/mL)	9.5283	1.0684	11.04	2.5642	0.212
T_{max} (h)	Median	Range	Median	Range	
	1.00	1.00~1.5	1.00	0.75~1.00	0.011

Table 1
Samples for E-tongue analyses.

No	Sample	FM	Abbreviation	Weight
1	FM API	Pure FM	FM	0.04 g
2	Physical mixture of optimal prescription	FM+E PO	PM-FM	0.8 g
3	Eudragit® E PO	Without FM	PM-Placebo	0.8 g
4	HME of optimal prescription	FM+E PO	H-FM	0.8 g
5	HME of Eudragit® E PO	Without FM	H-Placebo	0.8 g
6	FM commercial granule	With FM	G-FM	0.8 g
7	FM ODT	With FM	O-FM	0.8 g
8	FM taste-masked ODT	With H-FM	O-H-FM	0.8 g
9	ODT	Without FM	O-Placebo	0.8 g

Table 2
The result of variance analysis.

Source of variation	SS	df	MS	*F*	*P*	Significance
Temperature	4.55	2	2.28	5.47	>0.05	Not
Speed	0.83	2	0.42	0.11	>0.05	Not
Loading	7.72	2	3.86	-2.1	>0.05	Not
Error	-3.67	2	-1.84			
Total variation	9.423	8				

Note: $F_{0.05}(2, 2) = 19$, $F_{0.01}(2, 2) = 99$; $P<0.05$, significant difference, $P<0.01$, extremely significant difference

enhanced the variations between groups and decreased the variations within groups. Both results proved the good smell-masked effect of O-H-FM. Compared with the E-tongue evaluation, the taste was the main limitation of FM.

3.3.3. *In vitro dissolution of ODT*

As shown in Fig. 13, ODT released less than 1.10% in 60 s in artificial saliva medium and more than 80% within 30 min in pH 4.5 buffer solution, which confirmed the taste-masked effect.

In short, taking together E-tongue, E-nose and *in vitro* dissolution, it has been adequately proven that FM ODT is sufficiently taste-masked.

3.3.4. Stability

Amorphous solid dispersions tend to recrystallize during storage, causing physical instability due to high-energy states. Therefore, it is necessary to select a proper matrix to form hydrogen bonds with the API, solving this problem and physically stabilizing the API over longer storage periods (Papageorgiou, 2009). The PXRD characterization and HPLC results showed that FM SD and ODT were physically and chemically stable during storage. These results showed that API was dispersed within the matrix and formed intermolecular interactions with Eudragit® E PO. In addition, the dissolution behaviour of FM ODT after 12 months of storage was similar (f2 = 86) to that of the fresh tablets, which indicated stability (Table 9).

3.4. Pharmacokinetics of the FM formulations in beagle dogs

The FM concentration in plasma was tested at various time points (Table 10). Concentration-time curves were generated (Fig. 14). The pharmacokinetic parameters were calculated (Table 11), and statistical analysis was performed (Table 12).

The T_{max} values of the two formulations were significantly different ($p>0.05$), 1.00 h and 0.75 h, respectively. This result indicated ODT had a slower absorption. FM granules were dissolved in water and then administered by gavage. This may lead to rapid absorption, with a smaller T_{max} and a larger C_{max}. FM ODT underwent water uptake, disintegration, dissolution, and absorption after administration. The particle size of the FM milled extrudate was 300~600 μm, which may lead to prolonged drug release and cause a larger T_{max} and a smaller C_{max}. However, $t_{1/2\lambda}$ and MRT were not significantly different, which may be due to the properties of FM. FM has a lower solubility in acidic media, so it is mainly absorbed in the intestine. FM ODT disintegrated quickly, and the matrix (Eudragit® E PO) dissolved at pH<5. Thus, these two formulations were mainly absorbed in the intestinal tract and had analogous distribution, supersession and elimination pathways, leading to similar $t_{1/2\lambda}$ and MRT values.

The values of the area under the concentration-time curve ($AUC_{0-\infty}$) of granules and ODT were 39.29 ± 6.5 μg•h/ml and 37.89 ± 3.79 μg•h/ml, and their F values were 86.23 ± 16.26% and 83.37 ± 11.91%, respectively, which indicated complete absorption and high bioavailability. (Tables 1 and 2)

4. Conclusion

Flunixin meglumine was successfully extruded with Eudragit® E PO by HME. Characterization studies proved the amorphous transition of FM. Then, direct compression was used to formulate the FM ODT. The main tablet properties were as follows: disintegration time of 17.6 ± 0.1 s and tensile strength of 0.7 ± 0.01 MPa. All the properties met the requirements of ODT. E-tongue, E-nose and dissolution analyses confirmed the taste-masked effectiveness. The pharmacokinetic study showed that FM ODT had pharmacokinetic behaviours similar to commercial granules in beagle dogs. In addition, a stability study proved the physical and chemical stability of FM SD and ODT.

In summary, FM ODT prepared in this study achieved a taste-masked effect and good pharmacokinetic behaviour and can be a potentially new formulation for clinical use. Moreover, there is a need to conduct further trials that involve target animals to ensure convenience, safety and efficacy of treatment.

CRediT authorship contribution statement

Yangfeng Xu: Conceptualization, Investigation, Formal analysis, Writing – original draft. **Guoqing Yan:** Conceptualization, Investigation. **Xuemei Wen:** Conceptualization, Investigation. **Liqin Wu:** Conceptualization, Investigation. **Ruihan Deng:** Conceptualization, Investigation. **Qiuling Liang:** Conceptualization, Investigation. **Linjie Zhang:** Conceptualization, Investigation. **Hangping Chen:** Conceptualization, Investigation. **Xin Feng:** Formal analysis, Writing – original draft, Writing – review & editing. **Jiakang He:** Conceptualization, Formal analysis, Writing – review & editing.

Declaration of Competing Interest

The authors have no conflicts of interest to disclose. Contributed reagents/materials/analysis tools: H.C. Wrote the paper: Y.X., J.H.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 31960717) and the Technology Research & Development Program of Nanning, Guangxi (No. 20175171). We wish to thank Dr. Xilong Xiao and Dr. Yafen Guo for their technical assistance.

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