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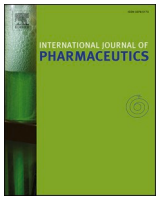

Developing an innovative 3D printing platform for production of personalised medicines in a hospital for the OPERA clinical trial

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ABSTRACT

Breast cancer is the most frequently diagnosed cancer in women worldwide, and non-adherence to adjuvant hormonotherapy can negatively impact cancer recurrence and relapse. Non-adherence is associated with side effects of hormonotherapy. Pharmacological strategies to mitigate the side effects include coadministration of antidepressants, however patients remain non-adherent. The aim of this work was to develop medicines containing both hormonotherapy, tamoxifen (20 mg), along with anti-depressants, either venlafaxine (37.5 or 75 mg) or duloxetine (30 or 60 mg), to assess the acceptability and efficacy of this personalised approach for mitigating tamoxifen side effects in a clinical trial. A major criterion for the developed medicines was the production rate, specified at minimum 200 dosage units per hour to produce more than 40,000 units required for the clinical trial. A novel capsule filling approach enabled by the pharmaceutical 3D printer M3DIMAKER 2 was developed for this purpose. Firstly, semi-solid extrusion 3D printing enabled the filling of tamoxifen pharma-ink prepared according to French compounding regulation, followed by filling of commercial venlafaxine or duloxetine pellets enabled by the development of an innovative pellet dispensing printhead. The medicines were successfully developed and produced in the clinical pharmacy department of the cancer hospital Gustave Roussy, located in Paris, France. The developed medicines satisfied quality and production rate requirements and were stable for storage up to one year to cover the duration of the trial. This work demonstrates the feasibility of developing and producing combined tamoxifen medicines in a hospital setting through a pharmaceutical 3D printer to enable a clinical trial with a high medicines production rate requirement.

1. Introduction

Breast cancer is the most frequently diagnosed cancer in women, causing the most deaths globally (Wilkinson and Gathani, 2022). Upon diagnosis, standard breast cancer therapy is based on chemotherapy, surgery, and radiotherapy, followed by a 5-to-10-year hormonotherapy maintenance phase (Trayes and Cokenakes, 2021). Adherence to the hormonotherapy in the maintenance phase is crucial for full recovery and prevention of relapse (Han, Wu et al. 2022).

Tamoxifen is the standard hormonotherapy in the maintenance phase for pre-menopausal women (Osborne, 1998). Tamoxifen is a prodrug mainly hepatically metabolised by the enzyme Cytochrome P450 2D6 (CYP2D6) to generate the active metabolite, endoxifen (Goetz, Kamal et al. 2008). Metabolism of tamoxifen is highly sensitive

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to CYP2D6 polymorphism, leading to great variation in endoxifen plasma levels and adverse drug reactions (Brooks, Comen et al. 2018, Chan, Law et al. 2020). Some of the most frequently experienced adverse effects include severe hot flashes, grade 2–3 musculoskeletal pain, and decreased libido (de Souza and Olopade, 2011). These can be severely disabling and are often a main reason for suboptimal tamoxifen adherence. Additional therapy with antidepressants is recommended to alleviate the side effects, such as venlafaxine for treatment of hot flashes and duloxetine for reduction of musculoskeletal pain (Boekhout, Vincent et al. 2011, Henry, Unger et al. 2018). However, low tamoxifen adherence remains an issue and is a major cause for early relapse and recurrence of breast cancer in recovering women (Mao, Hachem et al. 2020).

A recent clinical study carried out in a large cohort in France concluded that targeted interventions such as reduction of medicines administration to once daily and dose adjustments of tamoxifen and venlafaxine or duloxetine would increase adherence to tamoxifen therapy and thereby reduce breast cancer recurrence rates (Pistilli, Paci et al. 2020). Currently, tamoxifen tablets of 10 or 20 mg are prescribed as immediate release oral dosage forms (Dickschen, Willmann et al. 2012), whereas venlafaxine doses of 37.5 to 75 mg and duloxetine doses of 30 to 60 mg are prescribed as extended release and delayed release oral dosage forms, respectively (Nichols, Focht et al. 2011). Manufacturing combination dosage forms of tamoxifen and either venlafaxine or duloxetine through conventional methods is impractical due to both the release concerns and dose adjustments of each active pharmaceutical ingredient (API).

Three-dimensional (3D) printing is a transformative technology capable of producing bespoke medicines (printlets) with varying drug doses as well as products containing multiple APIs with different release requirements (Khaled, Burley et al. 2015, Keikhosravi, Mirdamadian et al. 2020, Awad, Hollis et al. 2023, Tracy, Wu et al. 2023, Yang, Stogiannari et al. 2023, Patel, Raje et al. 2024). As such, 3D printing offers a possible solution for targeted tamoxifen interventions such as differentiated doses and combination therapies to increase adherence among recovering breast cancer patients. Moreover, 3D printing offers a rapid method of production for small-batch medicines manufacturing, making it particularly suitable for personalised dosages and an enabler of clinical trials. To date, several 3D printing technologies for pharmaceuticals exist, with the most clinically advanced technologies being fused deposition modelling (FDM), direct powder extrusion (DPE), semisolid extrusion (SSE), binder jetting, and inkjet printing. Whilst implementation of 3D printing in hospital pharmacies is still in its infancy and challenges relating to technical feasibility and printing time are still being addressed (Annereau, Toussaint et al. 2021), a shift in treatment paradigm is anticipated and the first clinical studies assessing bioequivalence, treatment preferences, and printlet disintegration *in vivo* have already been published (Goyanes, Madla et al. 2019, Lyousoufi, Lafeber et al. 2023, Seoane-Viaño, Pérez-Ramos et al. 2023).

The aim of this work was to develop single dosage units comprising tamoxifen in 20 mg combined with either venlafaxine in 37.5 or 75 mg, duloxetine in 30 or 60 mg, or a placebo for usage in the OPERA clinical trial. Requirements for the developed medicines included release behaviour for each API (immediate tamoxifen release according to USP monograph for conventional tablets, and maintained extended venlafaxine and delayed duloxetine release), stability for up to one year of storage, as well as a minimum production rate of 200 dosage units per hour. To accomplish this, the M3DIMAKER 2 3D printer was employed as an innovative and versatile capsule filling platform. Firstly, the tamoxifen pharma-ink (printing feedstock) was deposited via SSE followed by the dispensing of venlafaxine or duloxetine pellets through a novel printhead. The printed medicines were produced in the department of clinical pharmacy in Gustave Roussy according to French compounding regulations and are part of the clinical trial OPERA (Odyssea PERsonalized Approach) which requires the production of more than 40,000 dosage units to investigate targeted tamoxifen interventions on patient adherence among 200 breast cancer patients. This study demonstrates the feasibility of producing multi-active dosage units containing tamoxifen through a pharmaceutical 3D printer in a hospital pharmacy at high production rates enabling a clinical trial to improve tamoxifen adherence in breast cancer patients.

2. Materials & methods

2.1. Materials

Tamoxifen citrate was provided by Hepartex® (Saint-Cloud, France). Duloxetine and venlafaxine were purchased from Lilly (Neuilly-sur-Seine, France) and Zentiva (Paris, France) companies, respectively. White/green size 1 capsules were provided by Cooper® (Melun, France). Polyethylene glycol 4000 (PEG 4000) Emprove® was provided by Merck (Darmstadt, Germany). Commercial dosage forms of tamoxifen, duloxetine, venlafaxine and DBCaps AAA masking were provided by Teva (Nanterre, France), Lilly (Neuilly-sur-Seine, France), Zentiva (Paris, France) and Lonza (Basel, Switzerland), respectively. 20-mL Injekt® syringes were supplied by B. Braun (Saint-Cloud, France), and polypropylene tips with an internal diameter of 1.53 mm were purchased from Fisnar® (Ellsworth, USA).

2.2. 3D printing technology selection

As one of the main requirements for the project was the production rate of minimum 200 dose units per hour, equivalent to 3.33 units per minute, a novel and rapid capsule filling approach enabled by SSE was successfully developed for tamoxifen. In addition, an innovative printhead for the M3DIMAKER 2 3D printer for accurate pellet dispensing was developed to enable rapid capsule filling with commercially available extended-release venlafaxine pellets, gastro-resistant duloxetine pellets, or placebo pellets.

2.2.1. Tamoxifen pharma-ink preparation and SSE optimisation

The SSE technology relies on the pressure-assisted extrusion of a semisolid pharma-ink through a syringe and nozzle. The semi-solid nature of the pharma-ink may be either dependent or independent of temperature. Here, SSE was employed as a capsule filing technology for tamoxifen loading into the capsules.

For the preparation of the pharma-ink, PEG 4000 (70% w/w) was heated at 75 °C for 1 h in a glass beaker placed in a water bath. Then, tamoxifen citrate (30% w/w) was incorporated by mechanical stirring at 800 rpm for 30 min with an electromechanical mixer from VMI (Montaigu, France) under the continued application of heat. The mixture was immediately transferred into 20 mL syringes and stored at room temperature until printing.

The SSE extrusion temperature was 75 °C and controlled using the software M3DIMAKER studio. Extrusion values ranging between 3.75 mm and 2.06 mm were investigated for the deposit of 101.1 mg of the pharma-ink, equivalent to 20 mg tamoxifen, in green and white size 1 capsules.

2.2.2. Dispensing of venlafaxine, duloxetine, and placebo pellets

The operating principle of the novel pellet dispensing printhead is that of an improved hopper with a moving element which displaces a precise quantity of pellets. The fill element contains a chamber of a specific diameter, which fills on the funnel side and empties on the capsule side. Different diameters of the fill elements were investigated to adjust the doses of venlafaxine to 37.5 and 75 mg and duloxetine to 30 and 60 mg. The deposited masses of pellets were calibrated by comparison to the pellet weights found in commercial dosage forms of duloxetine and venlafaxine.

2.3. Differential scanning calorimetry (DSC)

DSC measurements were performed with a Discovery X3 DSC (TA

Instruments, Waters, LLC, New Castle, DE, USA) to characterise the tamoxifen citrate powder, PEG 4000 powder, a powder blend of 30% w/w tamoxifen citrate and 70% w/w PEG 4000 (powder blend), and the pharma-ink containing 30% w/w tamoxifen citrate and 70% w/w PEG 4000. Approximately 5 mg of sample was accurately weighed out in Tzero aluminium pans and hermetically sealed with Tzero aluminium hermetic lids. Nitrogen was used as a purge gas with a flow rate of 50.0 mL/min. The samples were first equilibrated at 25 °C then heated at a rate of 10 °C/min from 25 to 200 °C. Thereafter, the samples were cooled at 10 °C/min from 200 to 25 °C, equilibrated at 25 °C, and heated again to 200 °C at a rate of 10 °C/min. Data were collected with TA TRIOS software (version 5.5.1.5, TA instruments, Waters LLC, New Castle, DE, USA) and analysed using TA Instruments Universal Analysis 2000.

2.4. X-ray powder diffraction (XRPD)

XRPD was performed on the raw powders of tamoxifen citrate and PEG 4000 as well as a powdered sample of the prepared pharma-ink (30% w/w tamoxifen citrate and 70% w/w PEG 4000) to investigate the solid state of tamoxifen in the pharma-ink. XRPD patterns were obtained with a Rigaku MiniFlex 600 (Rigaku, Wilmington, MA, USA) equipped with a Cu X199 ray source ($\lambda = 1.5418$ Å). Data acquisition was carried out between 3 and 60° 2θ with a stepwise size of 0.02° at a rate of 5°/min. The intensity and voltage applied were 15 mA and 40 kV.

2.5. High performance liquid chromatography (HPLC)

An Agilent 1260 Series HPLC system equipped with an online degasser, quaternary pump, column heater, autosampler and UV/Vis detector, was used. HPLC analyses were carried out by three different methods, depending on which of the three APIs were analysed. Table 1 summarises the three different HPLC methods used in terms of columns, column temperatures, mobile phase compositions, flow rates, mode of elution, injection volumes, and wavelengths for detection.

Analytical method validations were conducted according to ICH guidelines (Q2(R1) and Q3(B)).

2.5.1. Drug loading

Drug loading was carried out in triplicate for the tamoxifen pharma-ink. While still in the molten state, three samples of ca. 100 mg were sampled from different locations in the beaker and transferred to 20 mL volumetric flasks which were filled to q.s. with mobile phase. Drug loading was also assessed in triplicate for tamoxifen in the printed medicines in triplicate. The entire tamoxifen deposit of each capsule was placed inside a 20 mL volumetric flask which was filled to q.s. with mobile phase. All samples were analysed via HPLC as described in Table 1.

2.6. In vitro dissolution

In vitro drug release profiles were obtained using a USP-II apparatus AT Xtend 7 apparatus from Sotax (Saint-Louis, France). Dissolution buffers specified in European Pharmacopeia chapter 4.1.3 were used for the dissolution tests.

For tamoxifen, the developed medicines were placed in 1000 mL of 0.02 N hydrochloric acid (HCl) at pH 2 under constant paddle stirring (100 rpm) at 37 °C, as specified in the USP monograph for tamoxifen citrate tablets. 1 mL of sample was withdrawn at 0, 5, 15, 30, 45 and 60 min and replaced with HCl pH 2. The drug release was determined via HPLC according to Table 1. Dissolution tests were conducted in triplicate and were performed at time of production, and after 3 months storage in ambient conditions and accelerated stability conditions (40 °C and 75% relative humidity (HR)).

Tamoxifen *in vitro* dissolution was also assessed at pH 4.5 and 6.8 following the European Medicines Agency (EMA) guidelines for biowaiver for bioequivalence (EMA guideline CPMP/EWP/QWP/1401/98) comparing the developed medicines to commercial tamoxifen citrate tablets.

Commercial pellets of duloxetine or venlafaxine were used for the manufacturing process. Dissolution profiles of duloxetine and venlafaxine pellets within the developed medicines were compared to the commercial dosage forms to ensure the suitability of the manufacturing process using a USP-I apparatus AT Xtend 7 apparatus from Sotax (Saint-Louis, France). For duloxetine, the dissolution assay was carried out in two stages according to dissolution test 1 in the USP monograph for delayed-release duloxetine capsules: the first stage for 2 h in 1000 mL 0.1 N HCl (pH 1.2) at 37 °C (100 rpm) with 1 mL samples withdrawn at 120 min, followed by the second stage of 1.5 h in 1000 mL phosphate buffer pH 6.8 at 37 °C (100 rpm) with 1 mL samples withdrawn and medium replacement at 0, 15, 45, 60, and 90 min. For venlafaxine, dissolution test 11 in the extended-release venlafaxine hydrochloride capsules USP monograph was performed for 24 h in 900 mL phosphate buffer pH 6.8 at 37 °C. A modified sampling schedule was performed with 1 mL samples withdrawn and media replacement at 0, 2, 18, and 24 h with the first (2 h) and last (24 h) sample points as specified in the USP monograph. All samples were analysed via HPLC as per Table 1.

2.7. Stability indicating method

Forced degradation studies were conducted on each API contained in the developed medicines (tamoxifen, venlafaxine, and duloxetine)

Table 1
Chromatographic conditions for the three APIs in this study.

	Duloxetine	Tamoxifen	Venlafaxine
Column	Zorbax Eclipse XDB C8 5 µm 150 x 4,6 mm (Agilent, Les Ulis, France)	Hypersil ODS C18 5 µm 250 x 4,6 mm (ThermoFisher, Waltham, MA, USA)	Inertsil C8 5 µm 250 x 4,6 mm (GL Sciences, Tokyo, Japan)
Column temperature	40 °C	30 °C	30 °C
Mobile phase	A: 6.386 g of hexanesulfonate in 620 mL of water plus 150 mLof 2-propanol and 230 mL of acetonitrile B: acetonitrile	440 mg of sodium dihydrogenophoshate in dimethyl-octylamine (3.76 mL) + 490 mL of acetonitrile + 600 mL of water. Orthophosphoric acid QS pH 3	A/B: 75%/25% A: 11.41 g of ammonium phosphate in 1 L of acid water (pH 3) B: acetonitrile
Flow rate	1.0 mL/min	1.0 mL/min	0.7 mL/min
Mode	Gradient elution	Isocratic	Isocratic
Injection volume	5 µL	10 µL	13 µL
Detection wavelength	230 nm	240 nm	225 nm

under hydrolytic (HCl 0.1 N and NaOH 0.1 N), oxidative (H_2O_2 3%) and thermic (100 °C) conditions to determine degradation products. Sampling was carried out at days 2, 4 and 7. Degradation products were identified and quantified with a threshold of 0.05% via HPLC, as described in Table 1.

The developed medicines were placed in polyvinyl chloride-polyvinylidene chloride (PVC-PVDC) blister packaging and stored in PSC 600 temperature and humidity-controlled chambers (Hettich, Sérézin du Rhône, France) for stability testing. The storage conditions were ambient conditions at 25 °C and 50% HR and accelerated stability conditions at 40 °C and 75% HR. Tamoxifen content and stability in the medicines was assessed via drug loading as per section 2.5.1 at time of production and after 0.5, 1, 2, 3, and 6 months of storage and ambient and accelerated conditions. In addition, *in vitro* dissolution profiles of all three APIs were assessed following 3 months of ambient and accelerated storage conditions as per section 2.6. All medicines were tested in triplicate per storage condition and specified test.

Pharma-ink containing syringes were also subjected to stability testing at ambient conditions and tamoxifen content and stability was assessed at time of production and following 1, 3, 6, and 12 months of storage as per section 2.5.1.

2.8. Residual water content

Residual water in the tamoxifen deposit may be a cause for interaction (e.g. partial dissolution) between the tamoxifen deposit, the pellets, and the gelatine capsule shell. Residual moisture content in the 3D printed tamoxifen medicines was assessed by two methods, namely loss on drying and Karl Fisher titration, and both were carried out in triplicate.

Loss on drying was conducted according to European pharmacopeia chapter 2.2.32, and the water content expressed as a percentage (w/w). The developed tamoxifen capsules were opened, and the deposited tamoxifen pharma-ink was weighed using a precision balance (XPE 205 Deltarange, Mettler-Toledo, Viroflay, France) before and after 24 h incubation at 100 °C.

Karl Fischer is a potentiometric titration method based on the oxidation of sulphur dioxide in methanol to determine water content. The developed tamoxifen capsules were opened, and the deposited tamoxifen pharma-ink was weighed and placed inside the Karl Fischer titrator (TL 7800, Xylem Analytics, France) wherein iodine was added dropwise for the determination of water content.

3. Results and discussion

As the overall production rate was one of the main concerns for the medicines development, SSE was investigated as a rapid capsule filling approach for precise pharma-ink deposits. An SSE tamoxifen formulation was developed for this purpose containing 30% w/w tamoxifen citrate and 70% w/w PEG 4000, as this ratio was optimal in terms of flow rate and volume of pharma-ink deposit. 20 mg tamoxifen per capsule, equivalent to the dose of a commercial tablet and standard care, was targeted, equivalent to 101.1 mg of the developed pharma-ink. The optimal extrusion value for this mass of pharma-ink deposit in each capsule was 2.8 mm which resulted in a relative standard deviation (RSD) of 4.1%. The acceptances value from the European Pharmacopoeia chapter 2.9.40 for mass variation was calculated and was lower than the maximum acceptance criterion of 15.0. The masses of deposited pharma-ink by varying the extrusion value and thereby exerted pressure on the syringe plunger can be found in Table 2.

On the other hand, this SSE approach was disregarded for the subsequent deposits of venlafaxine or duloxetine, mainly due to their individual modified release requirements necessitating the use of either water or organic solvents for excipient solubilisation. As such, challenges associated with the potential dissolution of capsule shell, additional drying steps, and maximum tolerated organic solvent content safe for human intake would have been encountered.

Therefore, a novel printhead for the M3DIMAKER 2 pharmaceutical 3D printer was developed by FABRX, allowing the precise dispensing of pellets. The principle of the pellet dispenser is a hopper system with an interchangeable element containing a reservoir of a specific diameter. Changing the reservoir element enables the precise dispensing of pellets of various diameters and masses. Commercial pellets were fed into the reservoir of the printhead and subsequently deposited into the capsules. Calibration of the pellet dispenser printhead was carried out by comparing the masses of pellets within commercial duloxetine or venlafaxine dosage units and the masses of dispensed pellets through hopper reservoirs of varying diameters. Results for the mass calibration of

Table 3
Mass calibration of pellet dispenser printhead for commercial gastro-resistant duloxetine pellets. Values are mg of duloxetine pellets in commercial dosage units (left) and deposited commercial duloxetine pellets via different chamber diameters of the novel printhead (right). SD: Standard deviation; RSD: Relative standard deviation.

Commercial duloxetine units		Diameter (mm)	5.50	5.85	6.00
Capsule n°1	336.80	Deposit n°1	297.10	350.00	340.60
Capsule n°2	331.60	Deposit n°2	300.20	337.00	348.10
Capsule n°3	320.00	Deposit n°3	301.30	340.50	348.30
Capsule n°4	345.00	Deposit n°4	306.20	342.60	349.30
Capsule n°5	341.80	Deposit n°5	298.90	340.60	344.30
Capsule n°6	338.20	Deposit n°6	301.50	344.00	346.40
Capsule n°7	330.50	Deposit n°7	301.50	341.20	350.70
Capsule n°8	341.10	Deposit n°8	301.80	338.30	346.30
Capsule n°9	338.00	Deposit n°9	303.60	339.50	346.40
Capsule n°10	334.40	Deposit n°10	298.50	346.50	343.00
Mean	335.74		301.06	342.02	346.34
SD	6.76		2.48	3.72	2.88
RSD (%)	2.01		0.82	1.09	0.83

Table 2
Mass calibration for SSE printhead with developed tamoxifen pharma-ink expressed as mg deposits. SD: Standard deviation; RSD: Relative standard deviation.

Percentage of extrusion	100	95	90	85	80	75	70	65	60	55
Extrusion value	3.75	3.56	3.38	3.19	3.00	2.81	2.63	2.44	2.25	2.06
Capsule n°1	136	130	128	120	114	106	98	90	97	61
Capsule n°2	140	131	132	119	108	116	97	91	82	81
Capsule n°3	140	131	129	112	113	110	100	89	82	73
Capsule n°4	140	129	123	118	112	105	100	92	85	80
Capsule n°5	142	128	123	121	112	108	98	90	89	78
Capsule n°6	139	126	125	121	110	109	101	90	87	71
Capsule n°7	134	127	127	120	113	99	101	97	83	86
Capsule n°8	136	126	128	117	110	103	100	87	82	78
Capsule n°9	135	126	126	118	111	110	95	93	79	69
Capsule n°10	135	124	126	120	122	106	95	90	82	70
Mean	138	128	127	119	113	107	98.5	90.8	84.8	74.6
SD	2.65	2.27	2.61	2.54	3.58	4.35	2.16	2.55	4.89	6.82
RSD (%)	1.9	1.8	2.1	2.1	3.2	4.1	2.2	2.8	5.8	9.2

duloxetine pellets are summarised in Table 3. The dispensing precision of commercial duloxetine pellets via the novel printhead was equal or not worse to that found for the commercial dosage units, as expressed by the relative standard deviation of the pellet masses found in the commercial and the developed medicines.

Pharma-ink and pellet depositions were carried out sequentially in a staggered scheme for each batch. Deposition of the SSE tamoxifen pharma-ink first allowed for adequate solidification of the deposit before the addition of modified-release pellets, avoiding potential interactions between unsolidified pharma-ink and pellets. All batches of the printed medicines were prepared according to French regulation for compounding (*hospital compounding preparation and magistral, article L5121-1 of the French Code of Public Health*). Moreover, the capsule filling approach for the production ensures that a double-blind trial can be carried out with the developed medicines.

An overview of the 3D printer setup with both the SSE printhead for tamoxifen deposits and two different pellet dispensers can be seen in Fig 1A. In Fig 1B, a comparison of size 1 capsules filled with either tamoxifen pharma-ink deposit only, placebo pellets only, and tamoxifen deposit and placebo pellets can be seen, demonstrating the feasibility of containing the two components in the size 1 capsules.

Containing all targeted dose ranges for tamoxifen and duloxetine or venlafaxine within size 1 gelatine capsules are hypothesised to enhance adherence to treatment further, as compared to i.e. larger dosage forms. Fig. 2 compares one of the produced medicines to commercial forms of tamoxifen, venlafaxine, and duloxetine and demonstrates how the medicine burden may be reduced for the patients by replacing two daily dosage units with one of similar size. Moreover, the printed medicine is directly compared to a commercial masking capsule, which can be used to conceal multiple dosage units in one. This masking capsule comes in size AAA which is significantly larger than the size 1 capsules produced in this study, further increasing the likelihood of enhanced patient adherence. The dimensional differences between the commercial dosage units, the masking capsule, and OPERA developed medicines are also summarised in Table 4.

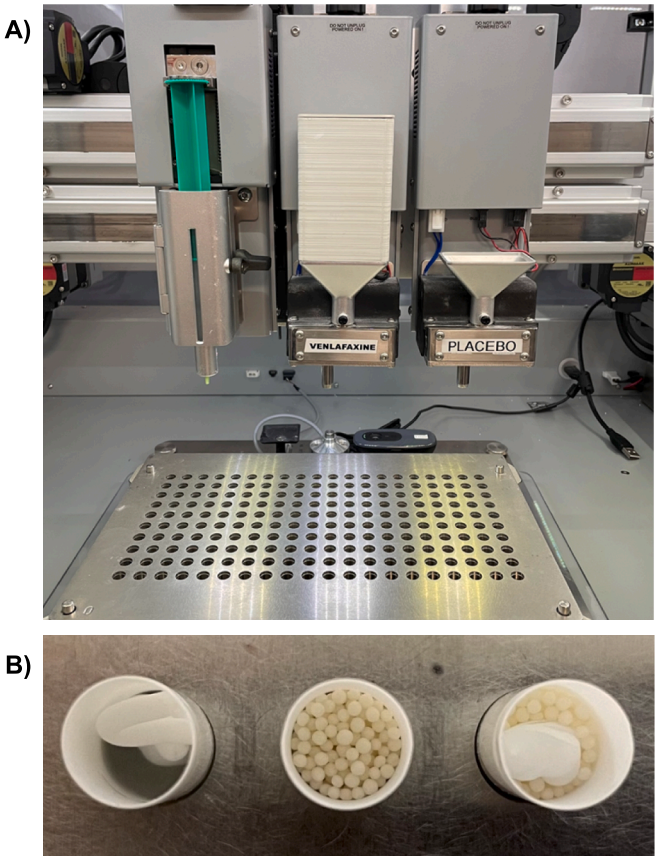

Fig. 1. A) Operating view of the SSE printhead for tamoxifen deposits and venlafaxine and placebo pellet dispenser printheads installed within the 3D printer at the same time, and B) Picture of (from left to right) size 1 capsule bottom filled with tamoxifen pharma-ink deposit only, placebo pellets only, and combination of tamoxifen pharma-ink deposit and placebo pellets.

A complete dosage unit, containing both tamoxifen pharma-ink deposit and venlafaxine, duloxetine, or placebo pellets, was filled in less than 10 s, and a whole batch of 200 units was filled in less than 26 min. A full batch of 200 units could be manufactured with a mean time of 45 min. This included the time required for preparation and analytical assessment of the pharma-ink, initialising and setting up the printer, filling the medicines, and post-production steps such as capping and analytical assessments. A schematic outlining the production process is presented in Fig. 3 below. The tamoxifen pharma-ink preparation step required the longest time of all steps. However, the pharma-ink could be produced in large batches, overall reducing its impact on the required manufacturing time. In the future, pharma-inks may be produced externally at licensed manufacturing sites, enabling a 'plug-and-play' compounding scenario for further reducing the required manufacturing time (Seoane-Viaño, Xu et al. 2023). This incredibly rapid production rate meant that a significant number of dosage units could be manufactured within a single day and before the commence of the trial, meaning it would be a suitable manufacturing method for the 40,000 dosage units required in the clinical trial.

DSC and XRPD analysis were performed to investigate the physical state of tamoxifen in the pharma-ink (Fig. 4). The sample of pure tamoxifen citrate showed a broadened melting endotherm at 147.67 °C with a slight shouldering at ca. 149.7 °C, indicative of the melting of two polymorphic forms (Fig. 4A). The diffractograms from the XRPD analysis confirmed that tamoxifen citrate was present as the polymorphic Form A, in both the pure tamoxifen citrate powder and the pharma-ink, confirmed by the comparison of unique diffraction patterns previously reported for the two polymorphic forms (Fig. 4B) (Goldberg and Becker, 1987). Therefore, it was confirmed that no alteration to the physical form of tamoxifen citrate was happening in the pharma-ink preparation. Resultingly, the second melting observed for the pure tamoxifen citrate powder in the DSC thermogram indicates the melting of Form B, after recrystallisation of the melted form A in the heating process of obtaining the thermogram. PEG 4000 powder showed a clear melting endotherm at 60.24 °C, and the powder blend and pharma-ink showed endothermic peaks at 60.45 °C and 59.17 °C corresponding to the melting of PEG 4000 (Fig. 4A). The pharma-ink preparation, involving the melting and subsequent solidification of PEG 4000, may have altered its thermic properties that resulted in the lowering of the observed melting endotherm by approximately 1 °C. For tamoxifen citrate, the melting endothermic peaks were now observed at slightly lower temperatures of 137.20 °C for the powder blend and 137.56 °C for the pharma-ink, both of which may be attributed to tamoxifen citrate dissolving partially in the polymer upon the heating (Medarević, Djuriš et al. 2019).

Shifting of a melting endothermic peak to a lower temperature for a crystalline API is known as melting point depression. The melting of a crystalline material happens when the free energy of fusion is zero, and the drug is present in both liquid and solid phases. In the liquid state, the drug displays an enhanced entropy which also holds true for any drug solubilised in the polymer. As such, upon heating, the solubilised drug will not exert any effect on the absorption of heat, thus leading to reduced drug enthalpy and thereby melting point. If the drug is insoluble in the polymer, no melting point depression should occur (Li, Tian et al. 2016, Moseson and Taylor, 2018).

Residual water content of the tamoxifen pharma-ink deposits was found to be very low. An average of 0.2% w/w residual moisture content was found with loss on drying (Table 5) and 0.412% w/w with Karl-Fischer titration (Figure S1). Low water content in the developed medicines ensures the absence of hydrolysis and degradation of the APIs due

Fig. 2. Pictures of (from left to right) commercial tamoxifen (white biconvex tablet), commercial duloxetine (blue/green size 2 capsule), commercial venlafaxine (pale pink size 2 capsule), commercial DBCaps AAA masking (opaque orange size AAA), and the developed medicine (green/white size 1 capsule). Scale in cm.

Table 4
Dimensional comparison of commercial medicines, masking capsule, and developed medicine.

Medicine	Tamoxifen tablets	Duloxetine capsules	Venlafaxine capsules	DBCaps® masking	OPERA medicines
Shape	Biconvex tablet	Size 2 capsule	Size 2 capsule	Size AAA	Size 1 capsule
Diameter	10 mm	–	–	–	–
Length	–	18 mm	18 mm	22.5 mm	22 mm
Height	4 mm	–	–	–	–
Volume	–	0.4 mL	0.4 mL	1.44 mL	0.5 mL

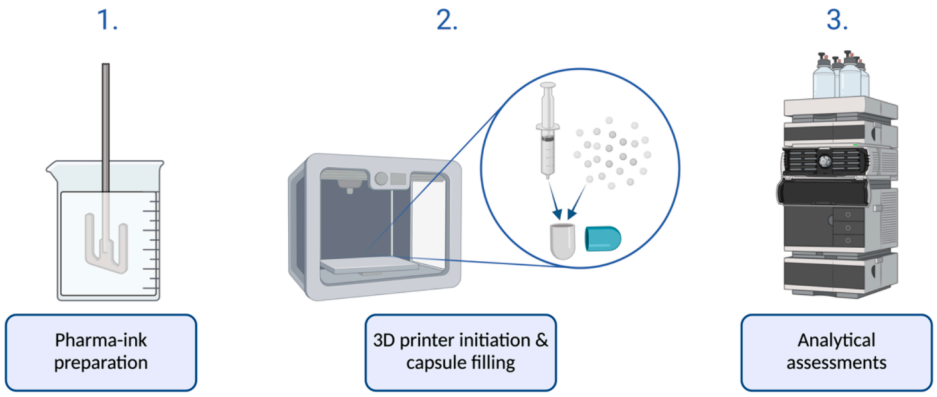

Fig. 3. Schematic of order of steps in manufacturing process for the developed medicines from pharma-ink preparation to analytical assessments. Figure created with BioRender.com.

to water presence (Zhou, Porter et al. 2017). These results further indicate the compatibility between the capsule shell and deposition method.

Dissolution tests were performed to confirm that the combination of tamoxifen with either duloxetine or venlafaxine in the produced capsules did not negatively influence the dissolution profiles of any of the three APIs. According to the USP monograph for tamoxifen citrate tablets, not less than 75% of the labelled amount should dissolve within 30 min in 0.02 N HCl at 37 °C under constant paddle stirring at 100 rpm. Dosage units from the pilot batch did not show any differences in the dissolution profile of tamoxifen in 0.02 N HCl as compared to the commercially available tamoxifen tablets when present alongside either venlafaxine or duloxetine (Fig. 5). For the developed medicines containing tamoxifen and venlafaxine (Fig. 5A), 75% of the tamoxifen citrate had dissolved after approximately 15 min both at time of production, 3 months post storage at ambient conditions (25 °C and 50% HR), and 3 months of storage under accelerated stability conditions (40 °C and 75% HR). Likewise, for the developed medicines of tamoxifen and duloxetine (Fig. 5B), no difference was observed in dissolution profiles for tamoxifen from units immediately after production, upon 3 months of storage at ambient conditions, and 3 months post storage at accelerated stability conditions. A small delay in tamoxifen release was observed for the earliest time points (5 and 15 min) for all developed medicines compared to commercial tablets which is likely the lag-time observed for breaking of the gelatine capsule shell. Nonetheless, for all stability conditions for the developed medicines, 75% tamoxifen dissolution was obtained within 30 min.

The obtained dissolution data in 0.02 N HCl confirmed that the developed OPERA medicines conformed to the specifications stated in the USP monograph for tamoxifen citrate tablets. Moreover, tamoxifen

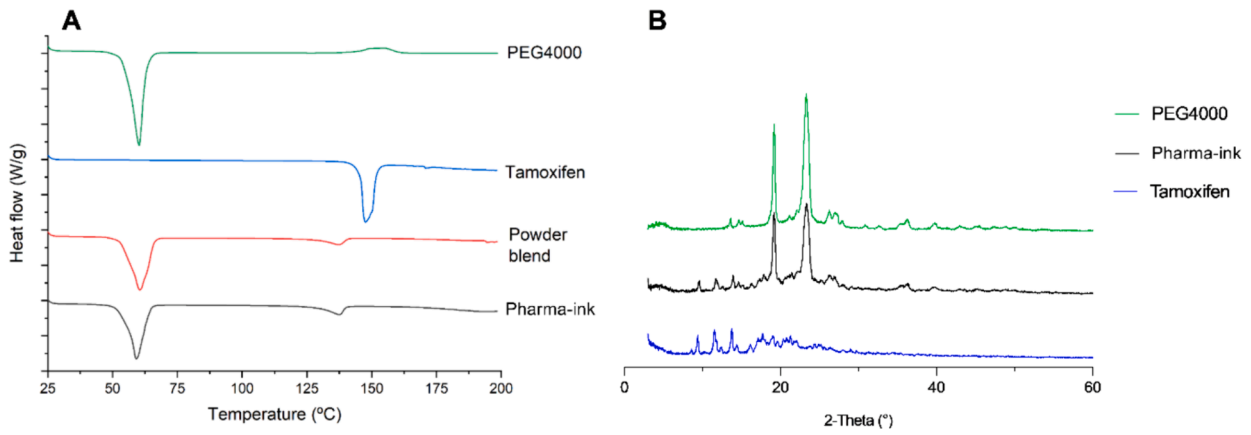

Fig. 4. (A) DSC thermograms (first heat cycle) of PEG 4000 powder, tamoxifen citrate powder ('tamoxifen'), powder blend, and tamoxifen citrate/PEG 4000 pharma-ink. (B) XRPD diffractograms of PEG 4000 powder, tamoxifen citrate powder ('tamoxifen') and tamoxifen citrate/PEG 4000 pharma-ink.

Table 5
Loss on drying results for tamoxifen pharma-ink deposits. Values are expressed as mass in mg at time of production (t_{0h}), after 24 h incubation at 100 °C (t_{24h}), and the difference between the two.

	Tamoxifen/duloxetine medicines			Tamoxifen/venlafaxine medicines		
n°	t_{0h}	t_{24h}	$t_{0h} - t_{24h}$	t_{0h}	t_{24h}	$t_{0h} - t_{24h}$
1	106.20	105.95	0.25	108.98	108.77	0.21
2	104.99	104.87	0.12	108.95	108.82	0.13
3	107.38	107.28	0.10	100.06	99.87	0.19
4	107.03	106.96	0.07	107.34	107.15	0.19
5	106.55	106.51	0.04	108.12	107.84	0.28
6	112.11	111.96	0.15	105.45	104.88	0.57
7	112.16	111.94	0.22	106.32	106.09	0.23
8	103.36	103.29	0.07	109.90	109.70	0.20
9	108.84	108.63	0.21	104.22	104.04	0.18
10	108.42	107.34	1.08	108.61	108.39	0.22
Mean	107.70	107.47	0.23	106.80	106.56	0.24

release from the developed OPERA medicines in media at pH 4.5 and 6.8 were comparable to commercial tamoxifen citrate tablets (data not shown). Tamoxifen is a Biopharmaceutics Classification System (BCS) Class II and EMA biowaiver and bioequivalence guidelines currently only apply to BCS Class I and III compounds. Therefore, the purpose of assessing *in vitro* dissolution across different media was not to submit a report for biowaiver on bioequivalence, rather it was to provide reassurance of the quality of the developed medicines compared to the commercially available tablets. As the highest doses of venlafaxine and duloxetine to be investigated in the clinical trial were used for the tamoxifen dissolution testing, no impact on tamoxifen release is expected for the lower doses of the two antidepressants. Since tamoxifen is a BCS Class II compound, the rate of absorption is governed by the drug solubility and/or dissolution rate. Therefore, there may be an initially slower absorption rate of tamoxifen from the developed medicines due to lag time for the capsule shell to disintegrate, as seen from the slower initial tamoxifen release in the *in vitro* dissolution tests (Fig. 5). However, the pharma-ink formulation of tamoxifen as a solid dispersion with PEG 4000 is expected to have equal or not worse bioavailability compared to commercial tamoxifen tablets as USP *in vitro* dissolution criteria are met. Plasma levels of tamoxifen and endoxifen will be monitored throughout the clinical trial to ensure safety and efficacy of the new tamoxifen formulation for the enrolled patients.

Dissolution data for venlafaxine and duloxetine pellets from the

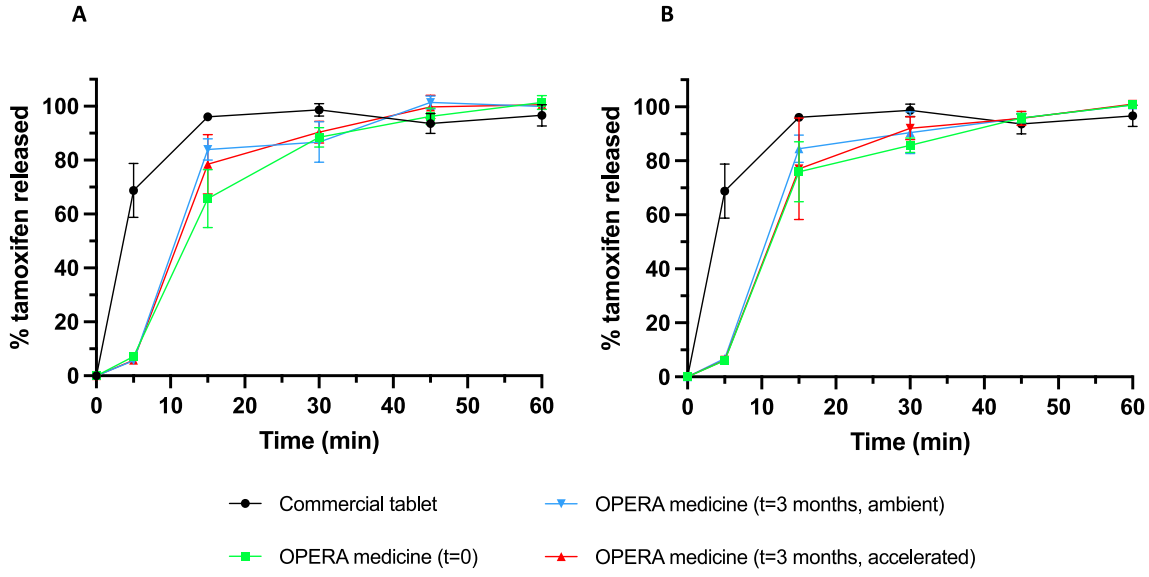

Fig. 5. Dissolution profiles in 0.02 N HCl of tamoxifen from the developed medicines also containing (A) duloxetine and (B) venlafaxine at time of production (green) as well as post 3 months of storage at ambient conditions (blue) and accelerated conditions (red). Reference groups were commercial tamoxifen citrate tablets (black).

developed medicines are presented in Figs. 6 and 7, respectively. For venlafaxine in the developed medicines, dissolution in phosphate buffer at pH 6.8 showed similar release profiles over the 24-hour testing period for all stability conditions of the produced medicines as compared to commercial dosage forms (Fig. 6). Less than 25% venlafaxine had released within 2 h whilst more than 90% of venlafaxine had released after 24 h in phosphate buffer pH 6.8, conforming to the early and end-point tolerances for the dissolution assay in the USP monograph.

Likewise, identical drug release profiles were observed for duloxetine from the developed medicines as compared to commercial dosage forms (Fig. 7). According to the USP monograph, not more than 10% duloxetine should be released from any dosage unit within the first 2 h in acid, whilst not less than 75% should dissolve within 90 min in pH 6.8 phosphate buffer. Both requirements were met for the developed medicines containing both tamoxifen and duloxetine.

Furthermore, all obtained dissolution data indicated that the developed medicines of both combinations would be stable upon storage for up to one year without compromising the dissolution characteristics of tamoxifen, venlafaxine, or duloxetine, supporting their use for the entire duration of the clinical study upon production. USP monographs were followed for *in vitro* dissolution requirements of finished dosage forms as no equivalent specifications are available in the European Pharmacopoeia.

The tamoxifen content of the developed dosage units at time of production and during the stability study remained within the acceptance limits of 95 – 105% of the labelled amount (i.e. 20 mg here) as specified in the European Pharmacopeia chapter 2.9.6 (Fig. 8A). Moreover, very low variation in tamoxifen content in the pharma-ink was observed during the stability study (Fig. 8B). No new degradation products appeared as consequence of the manufacturing process and storage for accelerated one-year storage conditions (Figure S2). USP impurities A (E-tamoxifen, retention time of 12.6 min) and F (retention time of 14.4 min) were identified upon capsule content analysis at time of production and post accelerated one-year storage (Figure S2). One unspecified and unknown product was identified at 3.9 min. The peak was integrated so that the product may be identified during routine analysis, however, the origin of this substance was not further investigated. No additional degradation products were identified in the printed medicines post accelerated one-year storage conditions.

A thermal stress study was conducted on tamoxifen citrate powder to assess its compatibility with heat processing. Tamoxifen remained stable at high temperatures, as seen from the absence of change in tamoxifen content over 7 days of incubation at 100 °C (Fig. 9). Moreover, the thermal stress study led to no formation of tamoxifen degradation products (Figure S3), confirming the suitability of the heat requiring pharma-ink and printing methods. Forced degradation studies of duloxetine confirmed that it was highly sensitive to acidic degradation as previously reported in literature, highlighting the necessity for a delayed release gastric-resistant formulation (Figure S4) (Sinha, Kumria et al. 2009, Chadha, Bali et al. 2016).

The developed tamoxifen medicines complied with the specifications of the European and U.S. pharmacopoeia in terms of dissolution testing and impurity assessment. The quality attributes of the developed medicines may be compared to those of the already commercially available dosage forms of tamoxifen, duloxetine, and venlafaxine. As such, the developed medicines were suitable for inclusion in the clinical trial based on both quality and production rate. All tests performed and obtained results have been included in the Investigational Medicinal Product Dossier (IMPD) submitted to The French National Agency for Medicines and Health Products Safety (ANSM) in connection with the clinical trial. Three previous clinical studies in patients have been reported with medicines produced via pharmaceutical 3D printing (Goyanes, Madla et al. 2019, Liu, Fu et al. 2023, Rodríguez-Pombo, de Castro-López et al. 2024). In all cases, the 3D printed medicines were associated with positive clinical outcomes. The OPERA clinical trial will be the largest clinical study to date with medicines produced through a pharmaceutical 3D printer in terms of participant numbers, and the clinical outcomes will be published once the trial has been completed.

4. Conclusion

A novel and rapid on-demand capsule filling approach enabled by a pharmaceutical 3D printer for developing tamoxifen therapies in combination with either duloxetine or venlafaxine was successfully developed for employment in the clinical pharmacy department at Gustave Roussy cancer hospital. The successful incorporation of immediate release tamoxifen, and either extended release venlafaxine or delayed release duloxetine was successfully obtained through SSE and a novel pellet dispensing printhead for the modified release APIs through the pharmaceutical 3D printer M3DIMAKER 2.

All batches were produced in accordance with French regulation for compounding, and all developed medicines met USP dissolution specifications and remained stable for up to one year of storage to cover the entire duration of the clinical study. No interactions were observed between tamoxifen, duloxetine, venlafaxine, and the capsule shell. The developed manufacturing method resulted in an average production time of 45 min for the entire production process of 200 dosage units, from pharma-ink preparation through to final tests, which satisfied the

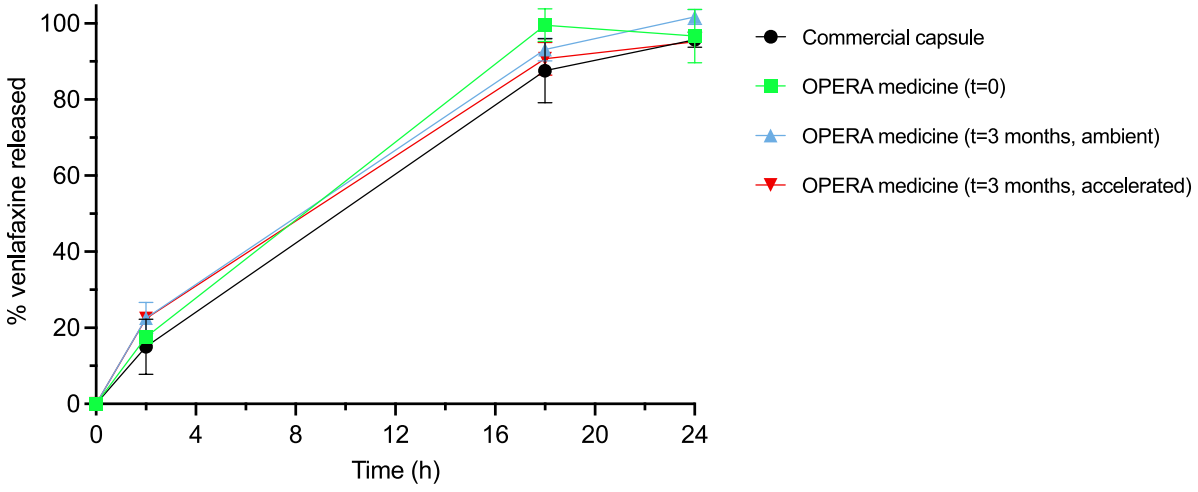

Fig. 6. Dissolution profiles of venlafaxine from the developed medicines in phosphate buffer pH 6.8 at time of production (green) as well as post 3 months of storage at ambient conditions (blue) and accelerated conditions (red). The reference group (black) was commercial venlafaxine capsules.

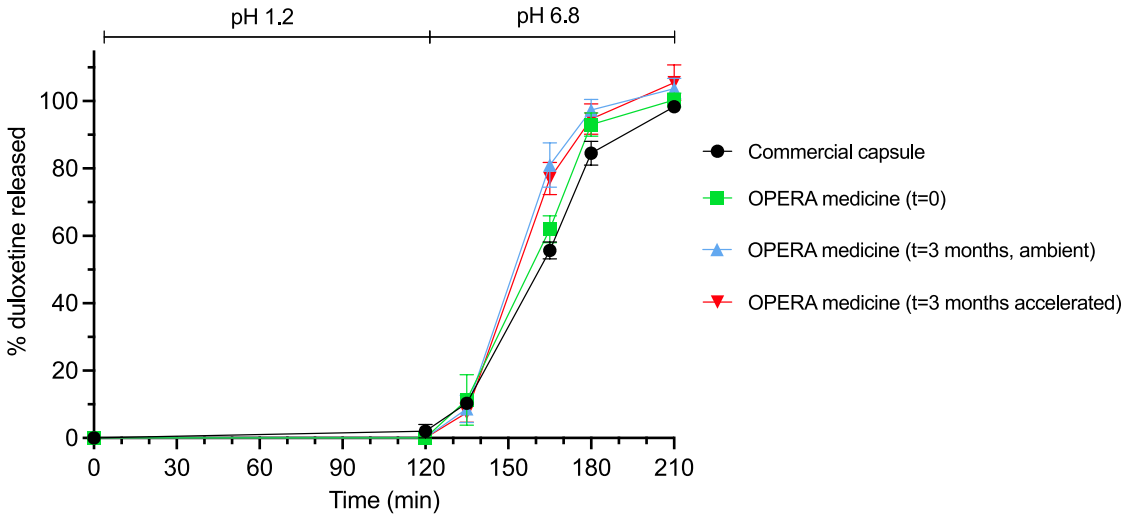

Fig. 7. Dissolution profiles of duloxetine from the developed medicines for 2 h in pH 1.2 followed by 90 min in phosphate buffer pH 6.8 at time of production (green) as well as post 3 months of storage at ambient conditions (blue) and accelerated conditions (red). The reference group (black) was commercial duloxetine capsules.

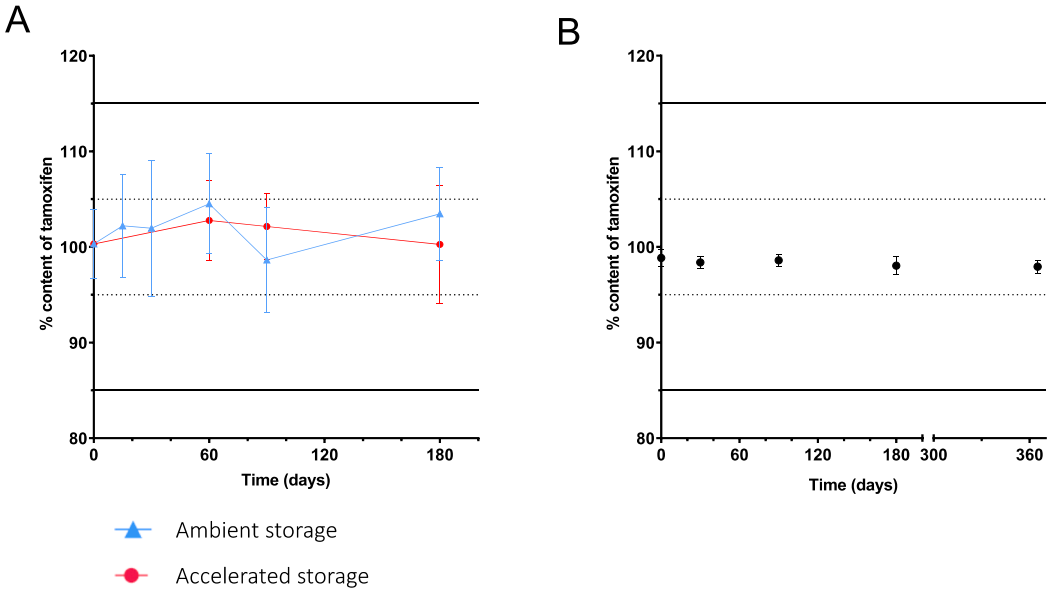

Fig. 8. (A) Tamoxifen content during 6 months of storage in the developed medicines at ambient conditions (25 °C and 50% HR) (blue graph) and accelerated conditions (red graph), and (B) tamoxifen content in pharma-ink during one-year stability study at ambient conditions.

predefined requirements.

This developed manufacturing method is part of the OPERA clinical trial to investigate the effect of reduced medicines intake on adherence rates to tamoxifen among recovering breast cancer patients. All tests performed and obtained results have been included in the IMPD submitted to ANSM to demonstrate the quality of the manufactured medicines to be administered to the patients in the clinical trial.

The successful development of combination therapies of tamoxifen and venlafaxine or duloxetine of flexible doses at very rapid production rates highlights the strength of pharmaceutical 3D printing as a versatile and flexible manufacturing technology enabling clinical trials and bespoke dosages.

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CRediT authorship contribution statement

Lucas Denis: Writing – original draft, Investigation, Formal analysis. **Anna Kirstine Jørgensen:** Writing – original draft, Methodology, Formal analysis. **Bernard Do:** . **Inès Vaz-Luis:** Supervision, Resources. **Barbara Pistilli:** Writing – review & editing. **André Rieutord:** Visualization, Resources. **Abdul W Basit:** Supervision, Resources, Conceptualization. **Alvaro Goyanes:** Writing – review & editing, Validation, Supervision, Methodology. **Maxime Annereau:** Writing – review & editing, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Abdul W. Basit reports a relationship with FABRX Ltd. that includes: employment and equity or stocks. Alvaro Goyanes reports a relationship with FABRX Ltd. that includes: employment and equity or stocks. If there

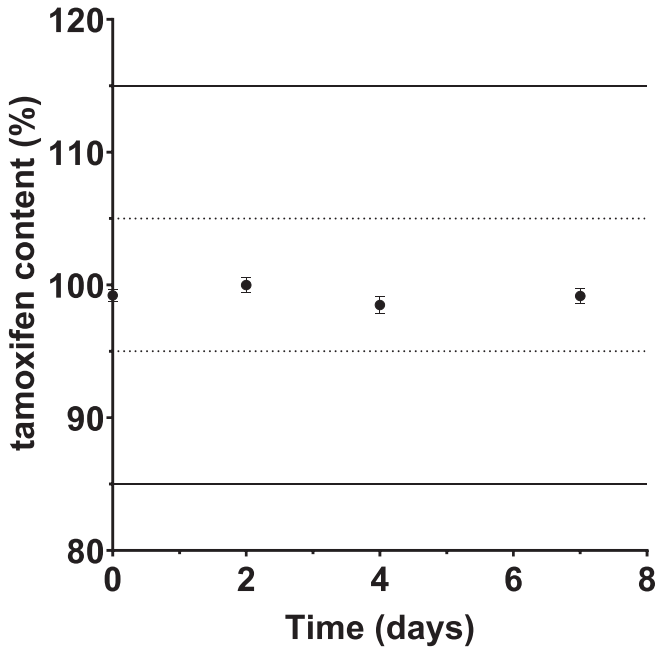

Fig. 9. Tamoxifen content in raw tamoxifen citrate material during the thermal stress study of 7 days incubation at 100 °C.

are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.].

Data availability

No data was used for the research described in the article.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2024.124306.

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