From ‘fixed dose combinations’ to ‘a dynamic dose combiner’: 3D printed bi-layer antihypertensive tablets.

Muzna Sadia[1], Abdullah Isreb[1], Ibrahim Abbadi[1], David Aziz[1], Peter Timmins[2], Mohamed A Alhnan[1]*

[1] School of Pharmacy and Biomedical Sciences, University of Central Lancashire, Preston, Lancashire, UK

[2] Department of Pharmacy, University of Huddersfield, Huddersfield, UK

*Corresponding author: MAlbedAlhnan@uclan.ac.uk

University of Central Lancashire, MB025 Maudland Building, Preston PR1 2HE, UK

Tel: +44 (0)1772 893590, Fax: +44 (0)1772 892929

A B S T R A C T

There is an increased evidence for treating hypertension by a combination of two or more drugs. Increasing the number of daily intake of tablets has been reported to negatively affect the compliance by patients. Therefore, numerous fixed dose combinations (FDCs) have been introduced to the market. However, the inherent rigid nature of FDCs does not allow titration of the dose of each single component for individual patient needs. In this work, flexible dose combinations of two anti-hypertensive drugs in a single bilayer tablet with a range of doses were fabricated using dual 3D printer. Enalapril malate (EM) and hydrochlorothiazide (HCT) loaded filaments were produced *via* hot-melt extrusion (HME). Computer software was utilized to design sets of oval bi-layer tablet of individualised doses. Thermal analysis and x-ray diffractometry (XRD) indicated that HCT remained crystalline in the polymeric matrix whilst EM was in the amorphous form. The interaction between anionic EM and cationic methacrylate polymer may have contributed to a drop in the glass transition temperature (Tg) of the filament and obviated the need for a plasticiser. Across all tablet sets, the methacrylate matrix provided similar *in vitro* drug release profiles despite difference in dose and layer thickness. This dynamic dosing system maintained the advantages of fixed dose combinations while providing a superior flexibility of dosing range, hence offering an optimal clinical solution to hypertension therapy in a patient-centric healthcare service.

A R T I C L E I N F O

Key words: Rapid prototyping; hypertension; MDDS; personalised; patient-centred; additive manufacturing; multiple drug delivery system

1. Introduction

The use of fixed dose combinations (FDCs) to enhance or simplify the treatment and to enhance patient compliance is a common approach in the management of long term conditions e.g. type 2 diabetes, HIV and hypertension (Bangalore et al., 2007; Desai et al., 2013). The history of combining multiple drugs in fixed doses dates back to 1950s; where the first combination was lunched as combined antihypertensive treatment (Wofford, 1997). At present, hypertension is often treated using several multiple drug classes, that are clinically used as dual or triple combinations (Wan et al., 2014).

FDCs offer a myriad of benefits in treatment of hypertension; improving adherence (Castellano et al., 2014; Laurent et al., 2004) and reducing number of tablets intake. Hence, they facilitate a simplified streamlined schedule. Moreover, combining two or more therapeutics at lower doses can offer superior clinical output than single agent in maximal dose (Garber et al., 2003; Haak et al., 2012). In addition, FDCs can offer more cost-effective therapeutic option than monotherapy (Bell, 2013). For instance, FDCs has lower cost in comparison to individual drugs as the cost of manufacturing, packaging and distribution is lowered (Desai et al., 2013; Gupta and Ramachandran, 2016).

Although convenient, the system is often too rigid to accommodate to changing an individual patient's needs e.g. the dose titration is particularly challenging for FDCs in a clinical setup (Xu et al., 2012) . For instance, if the prescriber identified the necessity to adjust the dose of one component in the FDC, one commonly used solution is the replacement of FDC with two separate dosage forms. In general, the doses in FDCs are designed to cover general population and hence they are less capable to meet the needs of small number of patients (Sleight et al., 2006).

3D printing is an emerging platform that offers many benefits in case of medicines personalisation (Alhnan et al., 2016; Prasad and Smyth, 2016). For instance, a dosage form can be fabricated according to the individual patient's need with reduced number of steps involved in manufacturing while excluding the need of the expensive designated facilities (Skowyra et al., 2015). Extrusion based 3D printing is capable of making a polypill with distinct release profiles (Khaled et al., 2015a, d). Although the technique offers the advantage of operation at room temperature, it often requires a long printing and drying time (typically 25 min and 24 h per tablet respectively)(Khaled et al., 2015a). It also mandates a significant compromise

between the viscosity of extruded materials, the size of the nozzle and the resolution of the finished product.

The low cost and widely used FDM 3D printers, however, offered a new and more accessible opportunity for on-demand fabrication of a ready-to-use tablets/caplets, with flexible doses of drugs of extended release (Goyanes et al., 2014; Goyanes et al., 2015a; Pietrzak et al., 2015; Skowyra et al., 2015; Tagami et al., 2017) as well as immediate release profiles (Li et al., 2017; Okwuosa et al., 2016; Sadia et al., 2016).

Although dual 3D printing has been reported for multilayer or core-shell fabrication to achieve dual drug or enteric drug release (Goyanes et al., 2015c; Okwuosa et al., 2017), there has been no previous report for fixed combination dose control in dual printing. This might be a reflection of the significant challenges often associated with dual FDM 3D printing; compatibility of the two materials, adhesion of printed layers in addition to the co-ordination of printing heads. These contributed to the difficulty of controlling drug dose in these multi-drug dosage forms.

Controlling the dose of 3D printed tablets might also raise a challenge in single head FDM 3D printing. Hot melt extrusion (HME) has been used to compound a filament as feed for FDM 3D printing. Changing drug loading in based filaments, however, would significantly impact the plasticity as well as drug release pattern. As FDM 3D printing process is particularly sensitive to changes in plasticity and rheological properties of the filament, it is hence of paramount importance to craft a filament so the printer can fabricate structures of a similar release profile from wide range of doses.

Therefore, the aims of this work are: i) to engineer a filament of wide range of drug contents and high compatibility with FDM 3D printing, ii) to utilise a low cost dual FDM 3D printer to achieve a dynamic dose combinations of two hypertensive drugs i.e. hydrochlorothiazide (HCT) and enalapril maleate (EM), and iii) to maintain similar release profile from individual or bi-layered system.

2. Materials and methods

2.1.Materials

Tri-calcium phosphate (TCP), hydrochlorothiazide (HCT) and triethyl citrate (TEC) were purchased from Sigma-Aldrich (UK). Enalapril maleate (EM) was acquired from Kemprotec Ltd. (Cumbria, UK). Eudragit EPO was donated by Evonik Industries (Darmstadt, Germany).

2.2.Preparation and optimisation of filaments

For *single HCT tablets,* drug loaded filaments were compounded with increasing percentages of HCT (X) (i.e. X= 0 (blank), 2.5, 5, 7.5, 10, 12.5, 25 or 50%). TCP (non-melting component) was employed as a substitute of HCT in each of the formulation to maintain incorporated solids content relative to the polymer. The final ratios of the formula were Eudragit EPO: TEC: HCT: TCP (46.75:3.25: X: 50-X), where X is the percentage of HCT in the filament. The nozzle size in this case was 1.25mm.

For bilayer tablets, two types of filaments were extruded containing either HCT or EM. HCT filaments (25% HCT) were produced at ratio: Eudragit EPO: TEC: HCT: TCP (46.75:3.25: 25: 25) as described above.

In case of EM, two filaments with different levels of plasticizer (TEC 3.25% and 2.5%) were initially produced using a 1.25 mm nozzle and ratios of EPO: TEC: EM: TCP 46.75:3.25:15:35 or 47.50:2.50:15: 35 respectively. However, these filaments were deemed too flexible and incompatible with FDM 3D printing process. Hence, a third filament with no plasticizer (without TEC) was produced at ratio EPO: EM: TCP 35:15:50 respectively using 1 mm nozzle head.

All filaments were then extruded using a HAAKE MiniCTW counter flow twin screw hot melt extruder (Karlsruhe, Germany). The materials were accurately weighed (10 g), mixed in pestle and mortar for 5 min, and then fed and extruded through HME at 100 °C.

2.3.Design and printing of tablets

Tablets were fabricated with HME based filaments using a dual FDM 3D printer, Makerbot Replicator 2X (Makerbot Industries, LLC, USA). The templates were designed using Autodesk® 3ds Max Design 2016 software version 18.0 (Autodesk, Inc., USA). The design was saved in a stereolithography (.stl) file format and was imported to the 3D printer's software, MakerWare Version Version 2.4.0.17 (Makerbot Industries, LLC, USA). Two sets of tablets were printed:

i) Single HCT tablets. In order to assess the release profile of the tablets of wide range of drug concentration, a series of tablet of identical dimensions (12 x 4.7 x 4.63 mm) has been printed for each HCT loading (2.5, 5, 7.5, 10, 12.5, 25 or 50%).

ii) HCT-EM Bi-layer. Six sets of bilayer tablets were fabricated with the combination of all doses, i.e. HCT: EM 25:5, 25:10, 25:20, 12.5:5, 12.5:10 or 12.5: 20 mg:mg. The Lower layer (first layer to be printed) was fabricated using EM filament while the upper layer was based on 25% HCT filament.

In order to achieve these doses, tablets of identical length and width (x=12 and y=6 mm) were printed while the height (z) was adjusted to allow the control of the printed volume of each layer and in turn its final mass and dose. The dose was calculated as:

$D_1 = 0.25\ W_1$ and $D_2 = 0.15\ W_2$

where D_1 and D_2 are the individual doses of HCT and EM, and W_1 and W_2 are the weight of layer containing 25% HCT or 15% EM respectively.

The tablets were printed (n=4) and a linear curve was plotted between the volume and mass of the tablets.

$$W_1 = D_1/0.25 = 1.4275\ V_1 - 5.5707$$

$$W_2 = D_2/0.15 = 1.5682\ V_2 - 8.1756$$

Where V_1 and V_2 are the volume of the HCT and EM layer in the bi-layer tablet.

The final dimensions of the layers to achieve the target dose are shown in **Table 1**.

2.4.Thermal analysis

Samples (5 mg) of raw materials and filaments were placed in TA aluminium pans 40 µL (standard) and pin holed lids and analysed using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). For DSC analysis, a differential scanning calorimeter DSC Q2000 (TA Instruments, Elstree, Hertfordshire, UK) was used. Samples were heated at a rate of 10 °C/min, from -50 to 280 °C preceded by a 1 min isotherm at -50 °C. The analysis was carried out under a purge of nitrogen at 50 mL/min. The collected data were analysed using a TA Universal Analysis 2000 v 4.5A software (TA Instruments, Elstree, Hertfordshire, UK).

TGA analysis was carried out using a TGA Q500 (TA Instruments, Hertfordshire, UK). Samples of raw materials and filaments (10 mg approx.) were placed in a 40µL aluminium pans and were heated from 25 to 500 °C at a rate of 10 °C/min under nitrogen flow of 60 mL/min.

In order to assess the impact of long term filament residence in hot nozzle during dual FDM 3D printing, drugs (as received) and filaments with HCT and EM at concentrations of 25% and 15% respectively were analysed by a thermo-scan from 25 to 140 °C with an isotherm of 30 min at the 3D printing temperature (135 °C). The changes in the mass of samples were then analysed using TA Universal Analysis 2000 v 4.5A software (TA Instruments, Elstree, Hertfordshire, UK).

2.5. X-ray diffractometer (XRD)

XRD analysis was carried out for raw materials, filaments and tablets using powder X-ray diffractometer, D2 Phaser with Lynxeye (Bruker, Germany). A scan was run from $2\theta = 7°$ to 50° with 0.1° step width and a 1s time count over 60 min. The divergence and scatter slits were 1mm and 0.6mm respectively. The X-ray wavelength was 0.154 nm using a Cu source, the voltage of 30kV and filament emission of 10 mA.

2.6.FT-IR Spectra measurements

FT-IR measurement for raw materials, filaments and tablets was carried out using Nicolet 5700 FT-IR spectrophotometer (Thermo Nicolet, Waltham, USA). The spectra were scanned between 4000 and 500 cm^{-1} at a resolution of 2 cm^{-1} using 128 accumulations/scan.

2.7.Characterisation of tablets

To analyse the impact of high temperature on drug after HME and 3D Printing, the tablets were assessed for drug content. Tablets (n=3) were randomly selected from each set and weighed. Each tablet was then placed in 1000 mL volumetric flask containing 0.1 M HCl and sonicated for 2 h. The solutions were then filtered using 0.22 mm Millex-GP syringe filters (Merck Millipore, USA) in a 1 mL vial. Simultaneous quantification was then carried out using an Agilent UV-HPLC 1260 series (Agilent Technologies, Inc., Germany) equipped with Kinetex C18 column (100 X 2.1 mm, particle size 2.6 mm) (Phenomenex, Torrance, USA). The mobile phase was acetonitrile: water (adjusted to pH 3 with o-phosphoric acid) and was measured at 230 nm. A gradient method was used for the quantification of drugs in HPLC (Water pH 3: acetonitrile 95:5 for 0-3 min, 95:50 to 50:50 from 3 to 8 min, 95:5 from 8.01 to 14 min) with a stop time of 14 min.

2.8.In vitro drug release from 3D printed tablets

In vitro drug release study was carried out using USP II Erweka DT600 dissolution tester (Erweka GmbH, Heusenstamm, Germany). For dissolution tests, tablets (n=3) from each of the sets: HCT: EM 5:12.5, 10:25 and 20:25 mg were placed in dissolution vessels containing 900 mL of 0.1M HCl. The paddle speed was set to 50 rpm while the temperature was maintained at 37 °C. The samples were collected at 0, 5, 10, 15, 20, 25, 30, 40, 50 and 60 min using 5 mL Leur-Lock syringes. The samples were then filtered out in 2 mL HPLC vial through Millex-HA 0.45 mm filters. The medium was then replenished with 4 mL of 0.1M HCl kept at same temperature. The quantitative analysis was then carried out using the HPLC protocol specified above.

3. Results and discussion

The proposed dynamic dose dispenser is based on dual 3D printer head, each nozzle is loaded with individual loaded filament and dose is controlled by varying the thickness of each layer in the tablet (**Fig. 1**). To achieve this, a universal filament system for immediate release (Sadia et al., 2016) was adapted to include two antihypertensive drugs: EM and HCT. Firstly, the flexibility of the method to comprise different concentration of HCT has been investigated by varying drug concentration from 2.5% to 50% w/w.

TGA studies showed no significant weight loss within the range of HME and 3D printing temperatures for all HCT filaments (**Fig. 2A**). DSC thermographs showed a Tg range of 22.9-35°C across all ratios of HCT and were compatible with FDM 3D printing process (**Fig 1B**). This suggests that the drug had a limited plasticising impact on filaments. As Eudragit EPO degrades >250 ^{o}C, it was not possible to detect the melting point of HCT (T_m= 267 ^{o}C) in both filament thermographs

XRD analysis were used to confirm the physical form of HCT in the filament **(Fig. 3)**, the diffraction peaks of HCT (as received) at 2theta = 16.65°, 19.13°, 20.95° and 24.64° were typical for HCT crystals (Khaled et al., 2015a). The presence of intensity peak at 2theta =19.13^{o} in all HCT filaments indicated that HCT remained in crystalline form after undergoing through the thermal processing of HME**.** The use of TCP as a complimentary filler to replace HCT allowed the production of filament of consistent properties. *In vitro* release drug from identical size tablets showed no significant difference of percentage of drug release at T=30 min (p>0.05) **(Fig. 4)**. This is of particular importance as it provides an alternative to controlling the dose of the 3D printed tablet by changing the size of the tablet, as previous reports indicated that surface area have a directly impact on its release pattern (Goyanes et al., 2015b; Pietrzak et al., 2015; Skowyra et al., 2015).

TGA of EM (as received) showed low weight loss within the temperature of HME and 3D printing processes (100 and 135 ^{o}C) (**Fig. 5A**). EM loaded filaments demonstrated a slight level of weight loss (up to 3%) across the different ratios of plasticiser. EM loaded filaments were initially produced using similar plasticiser concentrations of that used with HCT (TEC 2.5% and 3.25% w/w) and yielded filaments of glass transition temperature (T_g) values <15 °C (**Fig 5B**). This rendered the filament excessively flexible to be compatible with the FDM 3D printing procedure. Highly flexible filament is prone to frequent bending and deformation within gears that feed the hot nozzle of the FDM 3D printer. Since the drop in the Tg suggests that EM had

a plasticising effect on the methacrylic polymer, a new formulation was adapted by the removal of plasticizer (0 % TEC). By relying on drug's plasticizing capacity, the resultant filament showed a T_g of 50 ^oC (**Fig. 5B**), the latter proved more suitable for FDM 3D printing process.

On the other hand, the absence of melting peak of EM at 145 ^oC suggested that EM was in amorphous state within both the filament and the tablet. This suggest that amorphous form integrity of EM were maintained following the 3D printing process. XRD patterns of EM (as received) showed intensity peaks at 2theta= 5.2°, 10.41° and 20.65°, which are typical of EM crystals (Kiang et al., 2003). These peaks were absent in the filaments hence confirmed that the EM was in amorphous form within EM filament. The high level of miscibility of Eudragit E and EM might be related to the opposite charge of the anionic malate and the cationic polymer chains.

FTIR spectrum for Eudragit EPO showed peaks at 2770 and 2822 cm^{-1} corresponding to the absorption band of non-protonated dimethylamine of the polymer (**Fig. S1**), the filament showed a depression in these bands and hence suggest that EM neutralized the polymer. FTIR spectrum EM showed a band at 1750 cm^{-1} can be observed in both drug and physical mixture but disappeared in the filament and tablets. The band at 1750 cm^{-1} represents the stretching of C=O group (Ip and Brenner, 1987). The disappearance of this in the filament and tablets might indicate the carboxylic group of EM has interacted with the amino group of Eudragit EPO. These data indicate the cationic amino groups of Eudragit EPO and the carboxylic group of maleate (Ramirez-Rigo et al., 2014; Wang et al., 2004). Such an interaction between Eudragit E and anionic molecules has been used for the formation of molecules condensation (Guzman et al., 2012) and solid complexes (Quinteros et al., 2011; Ramirez-Rigo et al., 2014) with the purpose of optimizing drug release or enhancing oral bioavailability.

In dual FDM 3D printing, two thermal nozzles are orchestrated to extrude the filament in alternative fashion to fabricate multi-material structures. Hence while one filament is being processed through the nozzle, the other is held at elevated temperature in the second nozzle. Therefore, a retraction function has been introduced to many dual printing software to mitigate this effect. It is therefore important to assess the thermal stability of the filament at the printing temperature (135^oC) for a prolonged time. Both filaments were subjected to a 30-min isotherm at 135°C to analyse the impact of prolonged thermal exposure on weight loss. The data showed that both HCT as well as HCT filament were stable at this temperature without significant weight loss (**Fig 6 A1,A2)**. However, both EM (as received) and EM loaded filament showed

a weight loss under the same conditions **(Fig 6 B1,B2)**, which suggested that prolonged exposure of EM to an elevated temperature during dual 3D printing might result in thermal degradation. Hence, the lower layer in the bi-layer structure of the tablet (which was printed first) was chosen to contain EM in order to minimise the period of exposure of EM to an elevated temperature of the printing process.

Six sets of bilayer tablets with distinctive dose of EM and HCT were printed. The drug content of each layer were controlled by variating the thickness of individual drug layer within the structure **(Fig. 1 A).** Drug contents study was carried out in the filaments containing 25% HCT and 15% EM followed by 3D printed bilayer tablets **(Table 2)**. The target dose were achieved for the majority of the tablet sets, however, increased deviation was noted particularly in low strength tablets. Such variation could be mitigated by improving the consistency of the mixing and applying a tight control on the filament diameter.

The design of the bilayer tablets and an image of an exemplar tablet are shown in **Figs. 7A, B**, SEM images indicated that both drug layers were composed of 200 μm layer (**Figs. 7C,D**). It is interesting to highlight that HCT layer in the tablet was dominated with visible pores and embedded particles, while EM layers had a smoother surface and were more fused. On the other hand, Raman imaging indicated the consistent distribution of the drugs within each layer (**Fig. 7E**).

In vitro drug release pattern from 3 sets of bilayer tablets are shown in **Fig. 8**, all the tablets released over 85% of both drugs within the first 30 min. Although the methodology is not identical, the release pattern is comparable to compendial requirements (Convention, 2007). It is interesting to note, however, that despite the distinctive difference in solubility of the two drugs [722mg/L (Yalkowsky and R.M., 1992) and 25 000 mg/L (Budavari et al., 1996) for HCT and EM respectively], the release for both drugs were similar. This could be attributed to that fact that drug release is mainly regulated by the erosion of methacrylate polymeric matrix. Following tablet contact with acid medium, the cationic polymer ionises and dissolves in the medium (Dierickx et al., 2012), hence allowing both drugs to be released at similar rates.

In these tablets, drug release was similar across different doses and layer thickness. The independence of drug release from drug loading is of particular advantage in practice, when the dose of one or both drugs in the bilayer tablet are sought to be modified without affecting drug release pattern and potentially therefore oral bioavailability. Patients who need secondary prevention post myocardial infarction, for instance, may need to increase their ACE inhibitor

while ensuring that the diuretic dose is kept constant (National Clinical Guideline Centre, 2013).

In summary, we have reported the fabrication of bilayer tablets to achieve a new option of hypertensive patients (dose combination with dynamic dosing system). In a future scenario, physicians will be able to modify the dose for instance in response to patient's clinical data while maintaining a single dose and without the need to change direction to the patient or request tablet splitting.

4. Conclusion

We demonstrated the use of dual 3D printing to achieve a dynamic dose dispensing. This dispensing system holds the advantages of fixed dosage combinations while offering a flexibility on dosing in a drug combination, hence ensuring that patient's individual needs are continuously fulfilled. Despite differences in model drug miscibility in the polymer base, FDM 3D-printing-compatible filaments were engineered *via* the manipulation of plasticiser level and the addition of inert non-melting component. This demonstrates the potential flexibility of the system.

The use of methacrylate based bi-layer tablet system offers covering a wide range of drug loading (within the filament) and was tolerant to changing dose and layer thickness without affecting drug release from the 3D printed bi-layer tablets. Each tablet was printed with dual drugs and distinct dose combinations. In the future, utilizing such a low cost dynamic dosing system for antihypertensive combinations can potentially improve clinical outcomes and patient's experience.

Acknowledgments

The authors would like to thank UCLAN Innovation Team for this support and Mrs Reem Arafat for her help with graphics design. The authors would also like to acknowledge the School of Medicine and Biomedical Sciences, Sheffield University for their support with µCT data.

Conflicts of interest M A Alhnan is the innovator in pending patent applications WO 2016038356 A1, P1607548.3 GB and GB 1519128.1 in the field of 3D printing of medicines.

List of Figures

List of Tables

Supplementary data

References

Alhnan, M.A., Okwuosa, T.C., Sadia, M., Wan, K.W., Ahmed, W., Arafat, B., 2016. Emergence of 3D Printed Dosage Forms: Opportunities and Challenges. Pharm Res 33, 1817-1832.

Bangalore, S., Kamalakkannan, G., Parkar, S., Messerli, F.H., 2007. Fixed-dose combinations improve medication compliance: a meta-analysis. Am J Med 120, 713-719.

Bell, D.S., 2013. Combine and conquer: advantages and disadvantages of fixed-dose combination therapy. Diabetes Obes Metab 15, 291-300.

Budavari, S., O'Neil, M., Smith, A., Heckelman, P., Obenchain, J., 1996. The Merck Index, 12th ed., Entry# 3605.

Castellano, J.M., Sanz, G., Penalvo, J.L., Bansilal, S., Fernandez-Ortiz, A., Alvarez, L., Guzman, L., Linares, J.C., Garcia, F., D'Aniello, F., Arnaiz, J.A., Varea, S., Martinez, F., Lorenzatti, A., Imaz, I., Sanchez-Gomez, L.M., Roncaglioni, M.C., Baviera, M., Smith, S.C., Jr., Taubert, K., Pocock, S., Brotons, C., Farkouh, M.E., Fuster, V., 2014. A polypill strategy to improve adherence: results from the FOCUS project. J Am Coll Cardiol 64, 2071-2082.

Centre, N.C.G., 2013. MI - secondary prevention, Partial update of NICE CG48 Methods, evidence and recommendations, available online: https://www.nice.org.uk/guidance/cg172/evidence/myocardial-infarction-secondary-prevention-full-guideline-pdf-248682925 (last accessed 30/9/2017).

Convention, U.S.P., 2007. The United States Pharmacopeia : USP30 : the National Formulary : NF25. United States Pharmacopeial Convention Inc., Rockville, Md.

Desai, D., Wang, J., Wen, H., Li, X., Timmins, P., 2013. Formulation design, challenges, and development considerations for fixed dose combination (FDC) of oral solid dosage forms. Pharm Dev Technol 18, 1265-1276.

Dierickx, L., Saerens, L., Almeida, A., De Beer, T., Remon, J.P., Vervaet, C., 2012. Co-extrusion as manufacturing technique for fixed-dose combination mini-matrices. European Journal of Pharmaceutics and Biopharmaceutics 81, 683-689.

Garber, A.J., Donovan, D.S., Jr., Dandona, P., Bruce, S., Park, J.S., 2003. Efficacy of glyburide/metformin tablets compared with initial monotherapy in type 2 diabetes. J Clin Endocrinol Metab 88, 3598-3604.

Goyanes, A., Buanz, A.B., Basit, A.W., Gaisford, S., 2014. Fused-filament 3D printing (3DP) for fabrication of tablets. Int J Pharm 476, 88-92.

Goyanes, A., Buanz, A.B., Hatton, G.B., Gaisford, S., Basit, A.W., 2015a. 3D printing of modified-release aminosalicylate (4-ASA and 5-ASA) tablets. Eur J Pharm Biopharm 89, 157-162.

Goyanes, A., Robles Martinez, P., Buanz, A., Basit, A.W., Gaisford, S., 2015b. Effect of geometry on drug release from 3D printed tablets. Int J Pharm 494, 657-663.

Goyanes, A., Wang, J., Buanz, A., Martinez-Pacheco, R., Telford, R., Gaisford, S., Basit, A.W., 2015c. 3D Printing of Medicines: Engineering Novel Oral Devices with Unique Design and Drug Release Characteristics. Mol Pharm 12, 4077-4084.

Gupta, Y.K., Ramachandran, S.S., 2016. Fixed dose drug combinations: Issues and challenges in India. Indian J Pharmacol 48, 347-349.

Guzman, M.L., Manzo, R.H., Olivera, M.E., 2012. Eudragit E100 as a Drug Carrier: The Remarkable Affinity of Phosphate Ester for Dimethylamine. Mol Pharmaceut 9, 2424-2433.

Haak, T., Meinicke, T., Jones, R., Weber, S., von Eynatten, M., Woerle, H.J., 2012. Initial combination of linagliptin and metformin improves glycaemic control in type 2 diabetes: a randomized, double-blind, placebo-controlled study. Diabetes Obes Metab 14, 565-574.

Ip, D.P., Brenner, G.S., 1987. Enalapril Maleate. Analytical Profiles of Drug Substances 16, 207-243.

Khaled, S.A., Burley, J.C., Alexander, M.R., Yang, J., Roberts, C.J., 2015a. 3D printing of five-in-one dose combination polypill with defined immediate and sustained release profiles. J Control Release 217, 308-314.
Khaled, S.A., Burley, J.C., Alexander, M.R., Yang, J., Roberts, C.J., 2015d. 3D printing of tablets containing multiple drugs with defined release profiles. Int J Pharm 494, 643-650.
Kiang, Y.H., Huq, A., Stephens, P.W., Xu, W., 2003. Structure determination of enalapril maleate form II from high-resolution X-ray powder diffraction data. J Pharm Sci 92, 1844-1853.
Laurent, C., Kouanfack, C., Koulla-Shiro, S., Nkoue, N., Bourgeois, A., Calmy, A., Lactuock, B., Nzeusseu, V., Mougnutou, R., Peytavin, G., Liegeois, F., Nerrienet, E., Tardy, M., Peeters, M., Andrieux-Meyer, I., Zekeng, L., Kazatchkine, M., Mpoudi-Ngole, E., Delaporte, E., 2004. Effectiveness and safety of a generic fixed-dose combination of nevirapine, stavudine, and lamivudine in HIV-1-infected adults in Cameroon: open-label multicentre trial. Lancet 364, 29-34.
Li, Q., Wen, H., Jia, D., Guan, X., Pan, H., Yang, Y., Yu, S., Zhu, Z., Xiang, R., Pan, W., 2017. Preparation and investigation of controlled-release glipizide novel oral device with three-dimensional printing. Int J Pharm 525, 5-11.
Okwuosa, T.C., Pereira, B.C., Arafat, B., Cieszynska, M., Isreb, A., Alhnan, M.A., 2017. Fabricating a Shell-Core Delayed Release Tablet Using Dual FDM 3D Printing for Patient-Centred Therapy. Pharmaceutical Research 34, 427-437.
Okwuosa, T.C., Stefaniak, D., Arafat, B., Isreb, A., Wan, K.W., Alhnan, M.A., 2016. A Lower Temperature FDM 3D Printing for the Manufacture of Patient-Specific Immediate Release Tablets. Pharm Res 33, 2704-2712.
Pietrzak, K., Isreb, A., Alhnan, M.A., 2015. A flexible-dose dispenser for immediate and extended release 3D printed tablets. Eur J Pharm Biopharm 96, 380-387.
Prasad, L.K., Smyth, H., 2016. 3D Printing technologies for drug delivery: a review. Drug Dev Ind Pharm 42, 1019-1031.
Quinteros, D.A., Manzo, R.H., Allemandi, D.A., 2011. Interaction Between Eudragit (R) R E100 and Anionic Drugs: Addition of Anionic Polyelectrolytes and Their Influence on Drug Release Performance. J Pharm Sci-Us 100, 4664-4673.
Ramirez-Rigo, M.V., Olivera, M.E., Rubio, M., Manzo, R.H., 2014. Enhanced intestinal permeability and oral bioavailability of enalapril maleate upon complexation with the cationic polymethacrylate Eudragit E100. Eur J Pharm Sci 55, 1-11.
Sadia, M., Sosnicka, A., Arafat, B., Isreb, A., Ahmed, W., Kelarakis, A., Alhnan, M.A., 2016. Adaptation of pharmaceutical excipients to FDM 3D printing for the fabrication of patient-tailored immediate release tablets. Int J Pharm 513, 659-668.
Skowyra, J., Pietrzak, K., Alhnan, M.A., 2015. Fabrication of extended-release patient-tailored prednisolone tablets via fused deposition modelling (FDM) 3D printing. Eur J Pharm Sci 68, 11-17.
Sleight, P., Pouleur, H., Zannad, F., 2006. Benefits, challenges, and registerability of the polypill. Eur Heart J 27, 1651-1656.
Tagami, T., Fukushige, K., Ogawa, E., Hayashi, N., Ozeki, T., 2017. 3D Printing Factors Important for the Fabrication of Polyvinylalcohol Filament-Based Tablets. Biol Pharm Bull 40, 357-364.
Wan, X., Ma, P., Zhang, X., 2014. A promising choice in hypertension treatment: Fixed-dose combinations. Asian Journal of Pharmaceutical Sciences 9, 1-7.
Wang, S.L., Lin, S.Y., Chen, T.F., Cheng, W.T., 2004. Eudragit E accelerated the diketopiperazine formation of enalapril maleate determined by thermal FTIR microspectroscopic technique. Pharm Res 21, 2127-2132.
Wofford, J.L., 1997. History of fixed-dose combination therapy for hypertension. Archives of Internal Medicine 157, 1044-1044.
Xu, X.S., Yuan, M., Nandy, P., 2012. Analysis of dose-response in flexible dose titration clinical studies. Pharm Stat 11, 280-286.
Yalkowsky, S.H., R.M., D., 1992. Aquasol Database of Aqueous Solubility, College of Pharmacy, University of Arizona, Tucson, AZ

Table 1 Dimensions, volume and expected mass and dose of the HCT and EM layers in bi-layer tablets.

Dose	Dimensions			Volume (mm^3)	Expected weight (mg)	Expected dose EM (mg)	Expected dose HCT (mg)
	X (mm)	Y (mm)	Z (mm)				
Dose 1	12	6	1.6	90.5	112	-	24.6
Dose 2	12	6	0.8	45.24	55.93	-	12.5
Dose 1	12	6	0.6	33.9	41.9	5.53	-
Dose 2	12	6	1.1	62.2	76.9	10.2	-
Dose 3	12	6	2.2	124	154	20.3	-

Table 2 Layer dimensions, filament contents, target and achieved doses and dose efficiency of 3D printed tablets.

	Drug	Layer dimensions (X x Y x Z) (mm)	Filament content (%) ± SD	Target dose (mg)	Achieved dose (mg) AV± SD
Bilayer Table I	Enalapril layer	12 x6 x 0.6	94.42±0.56	5	4.93±0.56
	Hydrochlorothiazide layer	12 x6 x 0.8	92.35±2.45	12.5	11.73±0.36
Bilayer Table II	Enalapril layer	12 x 6 x 0.6	89.69±9.15	5	5.66±0.15
	Hydrochlorothiazide layer	12 x 6 x 1.6	91.98±2.73	25	25.82±0.68
Bilayer Table III	Enalapril layer	12 x 6 x 1.1	89.69±9.15	10	9.76±0.57
	Hydrochlorothiazide layer	12 x 6 x 0.8	91.98±2.73	12.5	12.95±1.07
Bilayer Table IV	Enalapril	12 x 6 x 1.1	89.69±9.15	10	9.64±0.13
	Hydrochlorothiazide	12 x 6 x 1.6	91.98±2.73	25	24.17±0.96
Bilayer Table V	Enalapril	12 x 6 x 2.2	96.98±1.39	20	20.11±0.64
	Hydrochlorothiazide	12 x 6 x 0.8	95.12±5.09	12.5	12.33±0.28
Bilayer Table VI	Enalapril	12 x 6 x 2.2	96.98±1.39	20	19.30±1.56
	Hydrochlorothiazide	12 x 6 x 1.6	95.12±5.09	25	24.85±3.03