Automated therapy preparation of isoleucine formulations using 3D printing for the treatment of MSUD: First single-centre, prospective, crossover study in patients

Alvaro Goyanes[a,b,*], Christine M. Madla[c], Aysha Umerji[c], Goretti Duran Piñeiro[d], Jose Maria Giraldez Montero[d], María Jesús Lamas Diaz[d], Miguel Gonzalez Barcia[d], Farhan Taherali[c], Paula Sánchez-Pintos[e], Maria-Luz Couce[e], Simon Gaisford[a,c], Abdul W. Basit[a,c,*]

[a] FabRx Ltd., 3 Romney Road, Ashford, Kent TN24 0RW, UK
[b] Departamento de Farmacología, Farmacia y Tecnología Farmacéutica, I+D Farma Group (GI-1645), Universidade de Santiago de Compostela, 15782, Spain
[c] UCL School of Pharmacy, University College London, 29–39 Brunswick Square, London WC1N 1AX, UK
[d] Pharmacy Department, Xerencia de Xestión Integrada de Santiago de Compostela, SERGAS, Travesía Choupana s/n, Santiago de Compostela 15706, Spain
[e] Servicio de Neonatología, Unidad de Diagnóstico y Tratamiento de Enfermedades Metabólicas Congénitas, Hospital Clínico Universitario de Santiago de Compostela, Universidad de Santiago de Compostela, IDIS, CIBERER, MetabERN, Spain

ARTICLE INFO

ABSTRACT

Maple syrup urine disease (MSUD) is a rare metabolic disorder with a worldwide prevalence of 1 in every 185,000 live births. However, certain populations display a significant overexpression of the disorder where incidence is reported to be 1 in every 52,541 new-borns. The first-line therapy for MSUD involves a strict dietary leucine restriction and oral supplementation of isoleucine and valine. The dose administered to patients requires strict tailoring according to age, weight and blood levels. In current clinical practice, however, practitioners still have to prepare extemporaneous formulations due to the lack of suitable oral treatments for MSUD. Herein, we evaluate the first time use of 3D printing in a hospital setting for the preparation of personalised therapies with the aim of improving safety and acceptability to isoleucine supplementation in paediatric patients suffering from MSUD. This investigation was a single-centre, prospective crossover experimental study. Four paediatric patients with MSUD (aged 3–16 years) were treated at the Clinic University Hospital in Santiago de Compostela, Spain which is a MSUD reference hospital in Europe. The primary objective was to evaluate isoleucine blood levels after six months of treatment with two types of formulations; conventional capsules prepared by manual compounding and personalised chewable formulations prepared by automated 3D printing. A secondary investigation was to evaluate patient acceptability of 3D printed formulations prepared with different flavours and colours. Isoleucine blood levels in patients were well controlled using both types of formulations, however, the 3D printed therapy showed mean levels closer to the target value and with less variability (200–400 μM). The 3D printed formulations were well accepted by patients regarding flavour and colour. The study demonstrates for the first time that 3D printing offers a feasible, rapid and automated approach to prepare oral tailored-dose therapies in a hospital setting. 3D printing has shown to be an effective manufacturing technology in producing chewable isoleucine printlets as a treatment of MSUD with good acceptability.

1. Introduction

Maple syrup urine disease (MSUD) is a rare metabolic disorder of autosomal recessive inheritance caused by the deficiency of branched-chain α-ketoacid dehydrogenase complex (BCKD). The defect in the catabolic pathway of branched chain amino acids (BCAA) – leucine, isoleucine and valine – result in toxic levels of BCAAs and their respective α-ketoacids in body fluids. MSUD has a worldwide prevalence of 1:185,000 live births, however, certain communities display an overexpression of this disorder including the Mennonite (Morton et al., 2002; Puffenberger, 2003) and Galician populations where the reported incidence is 1 in 52,541 new-borns (Couce et al., 2011). Leucine is the most neurotoxic BCAA where its elevation can induce encephalopathy leading to seizures, cerebral oedema, falling into a

---

coma and death (Lin et al., 2013; Nellis et al., 2003).

The first-line therapy for MSUD involves a strict dietary leucine restriction. Additional supplementation with isoleucine and valine in precise doses may be needed to avoid deficiencies (Enolia, 1992). The dose administered to each patient is tailored depending on age, weight and blood levels (Frazier et al., 2014a). In current clinical practice, there are a lack of suitable BCAA formulations available in the market and their administration requires the preparation of extemporaneous formulations that are firstly weighed out individually as a powder (Rodan et al., 2018) and dispersed in drinks or food (Frazier et al., 2014a).

Since MSUD is a life-long disease, designing the treatment preparation to be less time-consuming for hospitals and more acceptable for patients may lead to lower costs for healthcare providers. Increased acceptability and adherence to treatments can further reduce hospital admissions due to metabolic decompensations. However, the lack of systems to manufacture personalised medicines is a limitation to global pharmacy practice in general (Stegemann et al., 2016). This is of particular importance for paediatric patients where doses and formulation development require stringent observation (Liu et al., 2014).

Three-dimensional (3D) printing in the medical arena is an advanced technology used for the manufacture of surgical models and tools (Lee, 2016; Maruthappu and Keogh, 2014), tailored implantable devices (Wen et al., 2018; Zopf et al., 2013), cells in the scope of biomedical engineering and even organs (Kuehn, 2016; Michalski and Ross, 2014; Schubert et al., 2014). In the pharmaceutical industry, 3D printing has shown to be a revolutionary technology for the fabrication of personalised printlets (3D printed tablets) or drug loaded medical devices (Alhnan et al., 2016; Awad et al., 2018a; Awad et al., 2018b; Basit and Gaisford, 2018; Norman et al., 2017; Trenfield et al., 2018; Zema et al., 2017). One of the many 3D printing technologies available, semi-solid extrusion technology involves the preparation of a gel-like material which is deposited in layers through a nozzle onto a build plate (Firth et al., 2018; Vithani et al., 2019). One of its main benefits lies in the production of small batches of medicines, each with a tailored dosage (in addition to shapes, colour, design, flavour, sizes and drug release characteristics). Diseases which afflict a limited number of patients, therefore, may be a suitable niche application (Goyanes et al., 2019; Trenfield et al., 2018; Trenfield et al., 2019).

The aim of this study was to evaluate, for the first time, the suitability of using a 3D printing technology and software in a hospital setting to manufacture personalised treatments. Semi-solid extrusion 3D printing technology was used to prepare personalised chewable formulations of isoleucine and administered to four paediatric patients diagnosed with MSUD. The ability of the formulations to control isoleucine blood levels and patient acceptability were investigated.

2. Methods

2.1. Study design and participants

The study design was a single-centre, pilot, prospective, crossover investigation of two isoleucine formulations administered in outpatients with MSUD. The study was conducted at the Clinic University Hospital in Santiago de Compostela and approved by the Research Ethics Committee of Santiago-Lugo (2017/564). The study comprised of four patients (two females and two males) from 3 to 16 years of age diagnosed with and treated for MSUD in Galicia, Spain, who voluntarily took part in the study. A participant information sheet which stated that the data obtained during the study was to be used for research purposes was given to each patient. Additionally, a member of the research team verbally explained the purpose of the study and what it entails. A written consent was also obtained from parents or legal guardians. Prior to the study, the recruited patients were prescribed different doses of isoleucine with individual prescribing instructions depending on their BCAA blood levels (Table 1).

Table 1
Patient information.

Patient	Gender	Age (years–months)	Isoleucine dose (mg)	Prescribing instructions
1	M	5–0	50	Monday, Wednesday and Friday
2	F	3–8	100	Daily
3	M	16–1	200	Daily
4	F	10–1	100–150	Daily

The study was conducted for 6 months in total and divided in two stages;

i) For the first three months, the enrolled patients took their standard medication which were capsules filled with the correct dose of isoleucine. Formulations were prepared at the hospital by compounding (as detailed in Section 2.4).

ii) In the following three months, patients were administered chewable isoleucine printlets that were prepared at the hospital by semi-solid extrusion 3DP (as detailed in Section 2.5). The dose in the printlet formulation was adjusted by controlling the amount of material deposited by altering the computer 3D model.

Six types of chewable printlets of different flavours and colours were provided to each patient every two weeks according to flavour and colour as follows; (strawberry-red; orange-orange; lemon-yellow; raspberry-light blue; banana-light green and; coconut-black.

The 3D printed formulations were compared to the standard medication in terms of efficacy of maintaining isoleucine blood levels of the patients. Amino acids blood levels were obtained using a dried blood spot (DBS) that the parents of the patients sent to the hospital by post for analysis. Additionally, acceptability data of the formulations by the patients were collected via participant and parent reported outcome measures.

Treatment was held according to the Spanish MSUD Protocols where patients received a dietary BCAA restriction according to age and tolerance (Vitoria et al., 2018). The main objective was to maintain leucine concentrations below 300 µM and for isoleucine levels to be between 200 and 400 µM (concentrations, however, should not lower that of leucine) (Frazier et al., 2014b; Jouvet et al., 2005).

2.2. Analytical methods

Quantitative analysis of isoleucine was performed by MS/MS from dried blood spot (DBS) samples obtained from the patient at least every two weeks. Amino acid analyses from dried blood spot samples includes a preparative step of elution and deproteinisation with 3% trichloroacetic acid. The analysis was carried out by ion-exchange chromatography after deproteinisation of the sample with 5-sulfosalicylic acid and a post-column reaction with ninhydrin (Couce et al., 2015).

2.2.1. Statistical analysis

The sample size was insufficient to presume the normality in the data and the number of tests to determine isoleucine level differs for each patient and for each formulation. The median values of isoleucine levels were obtained for the standard and 3D printed formulations for each patient and were compared using Wilcoxon signed-rank test (OriginPro 2017, OriginLab corporation USA). The sample size was insufficient to presume the normality in the data and the number of tests to determine if isoleucine level differs for each patient and for each formulation.

2.3. Acceptability testing

During the evaluation of the 3D printed formulations, a set of 14 sample formulations were sent to each of the participants bimonthly. The printlets were printed with the same shape in the previously

Please select the face that is most applicable:

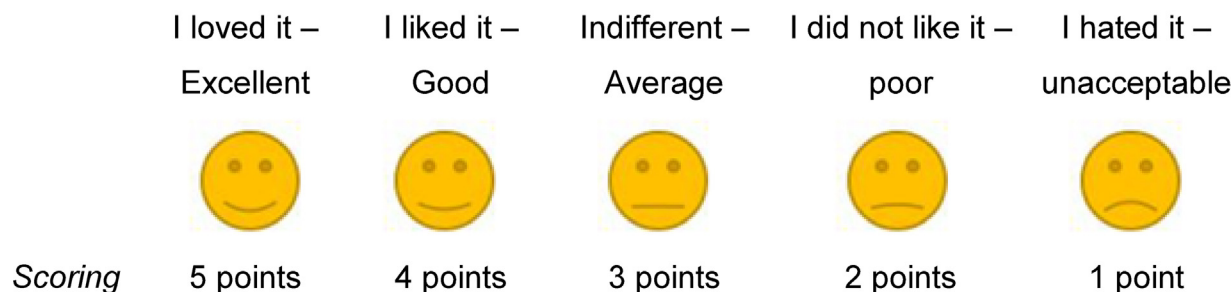| I loved it – Excellent	I liked it – Good	Indifferent – Average	I did not like it – poor	I hated it – unacceptable
Scoring 5 points	4 points	3 points	2 points	1 point

Fig. 1. 5-point facial hedonic scale, example of participant reported outcome.

indicated range of flavours and colours. The acceptability flavours and colours were evaluated using the five-point facial hedonic scale characterised with descriptions ranging from 5 = excellent to 1 = unacceptable (Fig. 1). Data was collected by the participant and parent reported outcome respectively (Goyanes et al., 2017).

One parent was present to observe the facial expression of the participant when taking each printlet and scored it from a scale of 1–3 ranging from signs of distress to signs of approval. Text examples of a parent reported outcome are as follows:

Please rate the participant's facial expression

o Positive face or other signs of approval (3 points)
o No facial expressions (2 point)
o Signs of distress (grimacing, "scrunching up" face, squinting eyes) or any other signs of disapproval (1 points)

The acceptability of the printlets were evaluated separately. Participant reported outcomes obtained from the five points hedonic facial scale were converted into numerical values (1 = unacceptable, 5 = excellent). Score values were then analysed using Kruskal-Wallis Anova (OriginPro 2017, Origin Lab corporation USA) to determine if there were any significant differences between the acceptability of the flavours and the standard formulations (p-value < 0.05).

2.4. Preparation of isoleucine capsules

The capsules of isoleucine were prepared by manual pharmaceutical compounding at the hospital following the standard operating protocol of the pharmacy department. The process involved the mixing of the isoleucine with a standard amount of microcrystalline cellulose for 30 min in an orbital mixer (Turbula) and manually filling the mixture in hard gelatine capsules. The ratio of isoleucine:microcrystalline cellulose was different in the preparation of capsules with different doses of isoleucine (50, 100, 150 and 200 mg).

2.5. Preparation of 3D printed chewable formulations (printlets)

Chewable printlets of isoleucine were prepared at the hospital incorporating 14.4% weight/weight (w/w) of isoleucine. Excipients used to prepare the chewable printlet formulations include sucrose, pectin, maltodextrin, water, flavourings and colourants. Six types of formulations with a different flavour and colorant were prepared including strawberry-red; orange-orange; lemon-yellow; raspberry-light blue; banana-light green and; coconut-black.

3D cylindrical printlets were prepared using a specially adapted 3D printer (The Magic Candy Factory, UK). Printlet fabrication included the loading of syringes with a mixture of excipients and isoleucine then heated to 70 °C in the printer to reach a viscosity suitable for fabricating the printlets by the extrusion. The 3D computer model used to print the formulations were designed with Autodesk 123D Design (Autodesk). This computer aided design program was used to design the 3D models

with 4 doses of isoleucine; 50 mg (8.2 mm diameter × 4.1 mm height); 100 mg (10.8 mm diameter × 5.4 mm height); 150 mg (12.5 mm diameter × 6.25 mm height) and; 200 mg (13.9 mm diameter × 6.95 mm height). 28 printlets were prepared per batch. Post-printing, the printlets were weighed individually and placed in Class B X-Large amber PVC blisters (Health Care Logistics, Inc. US).

The isoleucine load of the printlets was determined using LC-MS/MS (Agilent 2460, Agilent Technologies UK). Chromatographic separation was achieved using Synergi Hidro-RP 80A, 150 × 4.60 mm (Phenomenex, UK) column. The mobile phase consisted of water, acetonitrile and 0.1% formic acid. Quantitative values were obtained using MassHunter Workstation Qualitative Analysis Version B.06.00 (Agilent Technologies) by analysing chromatographic peak areas.

In vitro isoleucine printlet release profiles were determined using a USP-II dissolution apparatus (paddle speed 50 rpm and 37 ± 0.5 °C). Printlets were split into pieces to simulate chewing, placed at the bottom of the vessel and stirred in 900 mL water. During the dissolution test, 2 mL samples were manually removed at 5 min intervals and the percentage of amino acid release to the media was analysed by LC-MS.

For stability testing, isoleucine printlets were weighed individually and stored in blisters used in the study. The blisters were kept at 40 °C temperature/75% relative humidity to mimic accelerated stability tests. Printlets were weighed after 4 weeks storage and isoleucine content was analysed using LC-MS to determine whether the mean isoleucine loading of the printlets were different than the theoretical loading after processing or storage (stability).

3. Results

The chewable printlets were prepared in the hospital using a 3D printer. Six different formulations were prepared with different flavours and colours (Fig. 2). All were manufactured within specification; the formulations contained 14.11 ± 0.35% (w/w) isoleucine, disintegrated and rapidly released the amino acid within 5 min under simulated gastrointestinal conditions. All printed formulations remained stable on storage for one month under elevated conditions of temperature and humidity.

Four paediatric participants (3–16 years of age) were recruited and completed the study. Most of the patients received the same dose during the whole duration of the study. Patient 4, however, required an increased dose (from 100 mg to 150 mg) due to the patient suffering from metabolic decompensation from a cold. The results from the isoleucine blood levels for each patient during the study are shown in Fig. 3A.

All the isoleucine levels ranged between 50 and 400 µM for both printlets and standard capsules. Isoleucine blood concentrations obtained from the DBS are comparable between the two types of formulations (Fig. 3B). The mean and the median values obtained with the 3D printed formulations were 214.77 µM and 205.83 µM respectively, and 181.64 µM and 175.34 µM for the capsules. The values obtained with the 3D printed formulations were within the range of the target aim (200–400 µM) but this was not the case for capsules. The

Fig. 2. Chewable printlets in different flavours/colour and doses. From left to right: Lemon-yellow, coconut-black, banana-light green, orange-orange, raspberry-light blue, strawberry-red. From top to bottom: 50 mg, 100 mg, 150 mg and 200 mg. Units are cm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

interquartile range (IQR) for the 3D printed formulations was also smaller when compared with the capsules. However, there were no significant differences between the isoleucine levels of the patient treated with the standard formulations or printlets.

The parents of the participants completed a questionnaire regarding the opinions of their children on the printed formulations with respect to flavour and colour. All formulations scored between excellent and average independently of the flavour or colour (Fig. 4A). The most accepted flavour and colour was orange. A statistical Kruskal-Wallis Anova was carried out to determine if the difference between the acceptability of the flavours and the standard formulations were significant and it showed that there was no statistical evidence to suggest that the participants preferred or disliked any of the 6 flavours over any of the others probably due to the small number of participants.

Facial expression of the participant when taking each of the formulations were scored from a scale of 1–3 ranging from signs of distress to approval in the parents reported outcomes (Fig. 4B). The results show similar trends to the patient reported outcomes for the formulations. Strawberry, orange and lemon-based formulations were the most accepted and the raspberry-based formulations scored the least.

Patient 1 identified all the flavours of the formulations except blackberry. The most preferred printlet colours were light green and light blue. The parents dissolved the standard capsule formulation in water which achieved no signs of displeasure. Patient 2 preferred the strawberry flavour but negatively responded to the coconut and blackberry-based formulations. The patient preferred printlets that were orange and yellow in colour. Facial expressions observed by parents were positive except for the coconut formulation. The standard capsule formulations were administered through a gastrostomy button therefore flavour evaluations could not be assessed for patient 2 specifically. Patient 3 preferred the orange flavour with coconut rated the worst. The patient took the standard capsules dissolved in milk to mask the flavour. Patient 4 reported good palatability and acceptability with all flavour and colour formulations apart from the coconut-flavoured

printlet. Patient 4 was given an increased printlet dose from 100 mg to 150 mg during the study due to metabolic decompensations from a cold.

4. Discussion

This is the first study to assess the viability of preparing personalised 3D printed formulations in a hospital setting with the selected dose based on the blood concentration values of the patients. All the MSUD patients with controlled levels of isoleucine treated and managed in the hospital were invited to participate in this study. As MUSD is a rare metabolic disorder, recruiting more participants for the study was a challenge.

Mean isoleucine blood values for the printlets were in the optimum range of isoleucine levels (200–400 µM) (Jouvet et al., 2005). As such, 3D printing could be used as a reliable method to prepare innovative formulations with a personalised dose, obtaining the targeted blood levels of isoleucine. Blood levels of isoleucine were obtained from blood spotted and dried on a matrix (DBS), a technique that has been used since the 1960s in clinical chemistry for mostly new-born screening. Since then, many drugs including nucleic acids, small molecules and lipids have been successfully measured using DBS. The use of this pre-analytical approach represents an interesting alternative to classical venous blood sampling; however its routine use is very limited (Lehmann et al., 2013). The possibility of sending DBS samples directly from each patient's home to determine BCAAs levels was crucial in this disease and was proven to be a very effective method to increase patient supervision.

For the capsules, the interquartile range (IQR) of isoleucine blood levels were wider than that of the printlets. One possible explanation for this is that although the capsules are designed to be swallowed, only one patient swallowed the complete formulation. Two participants opened the capsule and mixed the content with food or drinks albeit Patient 2 needed capsule administration through a gastrostomy button.
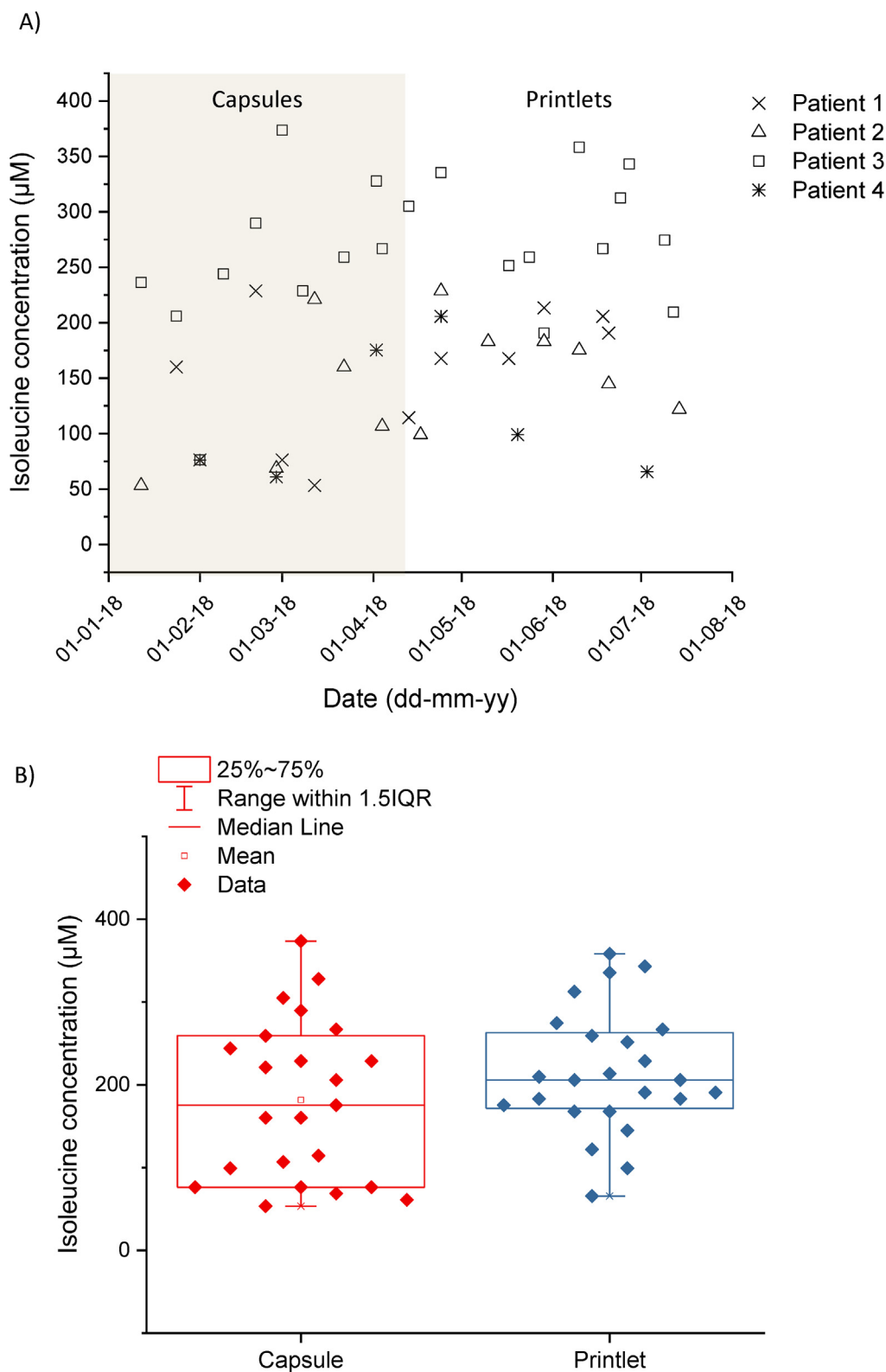
Fig. 3. A) Isoleucine blood levels of the participants during the study, B) Isoleucine blood levels and mean values for printlets and capsules during the study.

The administration of the isoleucine in that way may interact with the food and make the absorption and bioavailability more variable affecting the blood levels.

The 3D printed formulations are designed to be chewed and swallowed without the need of food or water and thus, may increase patient acceptability. The ingestion without food may also reduce the

variability of the absorption of the isoleucine. The printlets were well accepted by the children regarding flavour and colour with the orange-based formulation receiving the highest score from the patient reported outcome. Patients differed regarding most preferred formulation flavour and colour. Combined with the limited number of recruited patients due to the low prevalence of this disorder, it was not possible to
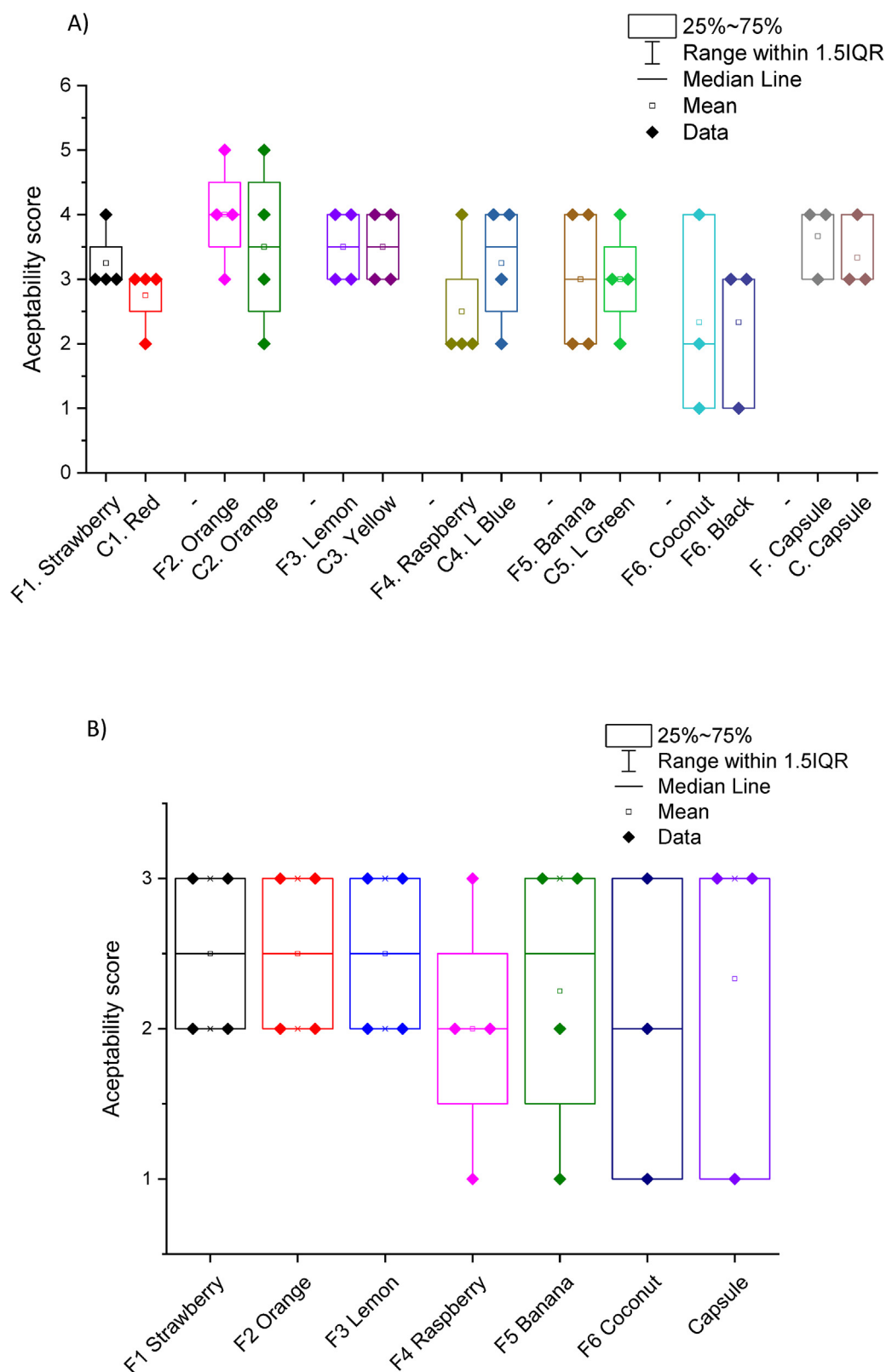
Fig. 4. A) Patient reported outcomes scores for the flavour and colour of the chewable printlets and the capsule. F and C refer respectively to the flavour and the colour of the formulations. B) Parents reported outcomes scores for the flavour and colour of the chewable printlets and the capsule. 1 point – signs of distress or any other signs of disapproval; 3 points – positive face or other signs of approval.

state that one flavour or colour was significantly more accepted that the other. However, it was possible to identify which flavour and colour combination were better accepted than the capsule. The coconut-black printlet was the worst rated formulation potentially because coconut

flavour is not a common or traditional flavour in the region of the study.

The one advantage of the 3D printing technology is that formulations are not limited to just one available flavour, and there is the potential to add a variety of flavours including the preferred by the

patient. This together with the fact that is possible to print different colours or shapes can make the medicines more appealing to the patients what may improve acceptability and compliance. The physical characteristics of palatability and texture alongside the size can also be optimised during the manufacturing process according to the preferences of the patient. It is also possible to prepare formulations avoiding the use of specific excipients that could cause allergic reactions to specific patients.

The preparation of the capsules following the specific requirements of the patient were obtained by pharmaceutical compounding. Compounding in hospitals and pharmacies serve an important role in modern health care to meet special patient care needs. This includes the discontinuation of commercially available products, limited dosage forms or strengths, unavailable drug products and combinations, new therapeutic approaches and special patient populations to name a few (Guharoy et al., 2013). Compounding is involved in approximately 10% of all prescribed medications in US (valued up to about $25 billion to $30 billion a year) (Allen, 2002). However, pharmaceutical compounding involves a series of serious and often severe risks as a single mistake in the daily practice may potentially result in patient maltreatment and even death.

Approved drugs in the market are manufactured in accordance to good manufacturing practice regulations (GMPs). In contrast, compounded drugs do not follow the same GMP regulations and testing to assess product quality is inconsistent (Gudeman et al., 2013). In the US, published reports have shown that compounded drugs fail to meet specifications at a higher rate than FDA-approved drugs (Gudeman et al., 2013). Most failures were related to potency (dose strength) ranging from 68 to 268% of the labelled dosage. The FDA concluded that compounding processes are most likely the cause of the quality failures and also reiterated that the rate of failure raises public health concerns for compounded drugs. Sub-potency is the most common susceptibility and some evaluations found that 34% of the formulations fell below the acceptable potency ranges prescribed by the United States Pharmacopeia (USP). Superpotency is less common but can have deadly consequences. Preparation contamination occurs when pharmacists manipulate drugs in nonsterile environments or by nonsterile means (Boodoo, 2010).

The automation of compounding using 3D printing could solve the previously cited problems of compounding. In this study, the formulations were in the range of 5% w/w weight variation. The automation of the compounding process not only increases the quality of the preparation regarding dose variation, it also keeps records of the whole process adding the capability of tracking the prepared formulations and thus, increasing safety by traceability. The printing of the isoleucine printlets is a fast process that allowed the fabrication of medication sufficient for one month (28 printlets) in approximately 8 min. The possibility of preparing formulations on-demand in a short period of time allows 3D printing to be more efficient, simpler and faster than traditional drug compounding. 3D printing can also reduce formulation contamination as all preparations occur inside the printer in an enclosed and contained space. In addition, the use of disposable build plates and ink cartridges significantly avoid any contamination problem.

This study has demonstrated the possibility to incorporate isoleucine, which currently has no licensed formulations on the market, into a chewable formulation using 3D printing. The dose strengths were prepared based on the normal dose given to the MSUD patients (50–200 mg) to maintain blood isoleucine levels between 200 and 400 μM (Frazier et al., 2014a), however higher doses have been also printed (500 mg). The versatility of the 3D printing technology paves the way for its use with other active materials and combinations of them (e.g. drugs or biologics). Furthermore, 3D printing is suitable for different types of formulations and not limited to chewable printlets. 3D printing not only allows the production of extemporaneous small batches of medicines in a short period of time but can further do so in an

automatic manner. Automation allows the tracking of the whole process and controlling all the possible variables, consequently avoiding errors and assuring higher quality standards. This technology, therefore, could be used for evaluation of new drugs in clinical trials when changing the dose is a requirement.

5. Conclusions

3D printing technology has shown to be successful in producing chewable printlets (3D printed tablets) of isoleucine as a treatment for MSUD. Mean and median isoleucine blood levels in patients following administration of the printlets were in the target range of 200–400 μM. There was good acceptability of the formulations by the patients although each patient had different preferences in terms of flavour and colour.

3D printing should be considered as an approach to prepare compounded medicines in a cost-effective, safe and automatic way in a hospital setting. The study demonstrates the first-time preparation and administration of 3D printed formulations in a clinical setting. As such, the 3D printing of pharmaceuticals can be used to advance the development of personalised medicines.

Declaration of Competing Interest

None.

Acknowledgements

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.

Allen, L.V., 2002. The Art, Science, and Technology of Pharmaceutical Compounding. American Pharmaceutical Association, Washington, DC.

Awad, A., Trenfield, S.J., Gaisford, S., Basit, A.W., 2018a. 3D printed medicines: a new branch of digital healthcare. Int. J. Pharm. 548, 586–596.

Awad, A., Trenfield, S.J., Goyanes, A., Gaisford, S., Basit, A.W., 2018b. Reshaping drug development using 3D printing. Drug Discovery Today 23, 1547–1555.

Basit, A.W., Gaisford, S., 2018. 3D Printing of Pharmaceuticals, 1 Ed. Springer International Publishing DOI: 10.1007/978-3-319-90755-0.

Boodoo, J.M., 2010. Compounding problems and compounding confusion: federal regulation of compounded drug products and the FDAMA circuit split. Am. J. Law Med. 36, 221–248.

Couce, M.L., Castiñeiras, D.E., Bóveda, M.D., Baña, A., Cocho, J.A., Iglesias, A.J., Colón, C., Alonso-Fernández, J.R., Fraga, J.M., 2011. Evaluation and long-term follow-up of infants with inborn errors of metabolism identified in an expanded screening programme. Mole. Genet. Metabol. 104, 470–475.

Couce, M.L., Ramos, F., Bueno, M.A., Diaz, J., Meavilla, S., Boveda, M.D., Fernandez-Marmiesse, A., Garcia-Cazorla, A., 2015. Evolution of maple syrup urine disease in patients diagnosed by newborn screening versus late diagnosis. Eur. J. Paediatric Neurol. 19, 652–659.

Enolia, T., 1992. Dietary management of inborn errors of amino acid metabolism with protein-modified diets. J. Child Neurol. 7, S92–S111.

Firth, J., Basit, A.W., Gaisford, S., 2018. The Role of Semi-Solid Extrusion Printing in Clinical Practice. In: Basit, A.W., Gaisford, S. (Eds.), 3D Printing of Pharmaceuticals. Springer International Publishing, pp. 133–151.

Frazier, D.M., Allgeier, C., Homer, C., Marriage, B.J., Ogata, B., Rohr, F., Splett, P.L., Stembridge, A., Singh, R.H., 2014. Nutrition management guideline for maple syrup urine disease: an evidence- and consensus-based approach. Mole. Genet. Metabol. 112, 210–217.

Goyanes, A., Allahham, N., Trenfield, S.J., Stoyanov, E., Gaisford, S., Basit, A.W., 2019. Direct powder extrusion 3D printing: fabrication of drug products using a novel single-step process. Int. J. Pharm. 567, 118471.

Goyanes, A., Scarpa, M., Kamlow, M., Gaisford, S., Basit, A.W., Orlu, M., 2017. Patient acceptability of 3D printed medicines. Int. J. Pharm. 530, 71–78.

Gudeman, J., Jozwiakowski, M., Chollet, J., Randell, M., 2013. Potential risks of pharmacy compounding. Drugs R&D 13, 1–8.

Guharoy, R., Noviasky, J., Haydar, Z., Fakih, M.G., Hartman, C., 2013. Compounding pharmacy conundrum: "We cannot live without them but we cannot live with them" according to the present paradigm. Chest 143, 896–900.

Jouvet, P., Hubert, P., Saudubray, J.M., Rabier, D., Man, N.K., 2005. Kinetic modeling of plasma leucine levels during continuous venovenous extracorporeal removal therapy in neonates with maple syrup urine disease. Pediatric Res. 58, 278–282.

Kuehn, B.M., 2016. Clinicians embrace 3D printers to solve unique clinical challenges. Jama 315, 333–335.

Lee, N., 2016. The Lancet Technology: 3D printing for instruments, models, and organs? Lancet 388, 1368.

Lehmann, S., Delaby, C., Vialaret, J., Ducos, J., Hirtz, C., 2013. Current and future use of "dried blood spot" analyses in clinical chemistry. Clin. Chem. Lab. Med. 51, 1897–1909.

Lin, N., Ye, J., Qiu, W., Han, L., Zhang, H., Gu, X., 2013. Application of liquid chromatography-tandem mass spectrometry in the diagnosis and follow-up of maple syrup urine disease in a Chinese population. J. Pediatr. Endocrinol. Metab. 26, 433–439.

Liu, F., Ranmal, S., Batchelor, H.K., Orlu-Gul, M., Ernest, T.B., Thomas, I.W., Flanagan, T., Tuleu, C., 2014. Patient-centred pharmaceutical design to improve acceptability of medicines: similarities and differences in paediatric and geriatric populations. Drugs 74, 1871–1889.

Maruthappu, M., Keogh, B., 2014. How might 3D printing affect clinical practice? BMJ 349, g7709.

Michalski, M.H., Ross, J.S., 2014. The shape of things to come: 3D printing in medicine. JAMA 312, 2213–2214.

Morton, D.H., Strauss, K.A., Robinson, D.L., Puffenberger, E.G., Kelley, R.I., 2002. Diagnosis and treatment of maple syrup disease: a study of 36 patients. Pediatrics 109, 999.

Nellis, M.M., Kasinski, A., Carlson, M., Allen, R., Schaefer, A.M., Schwartz, E.M., Danner, D.J., 2003. Relationship of causative genetic mutations in maple syrup urine disease with their clinical expression. Mole. Genet. Metabol. 80, 189–195.

Norman, J., Madurawe, R.D., Moore, C.M., Khan, M.A., Khairuzzaman, A., 2017. A new chapter in pharmaceutical manufacturing: 3D-printed drug products. Adv. Drug Deliv. Rev. 108, 39–50.

Puffenberger, E.G., 2003. Genetic heritage of the Old Order Mennonites of southeastern Pennsylvania. Am. J. Med. Genet. Part C: Seminars Med. Genet. 121C, 18–31.

Rodan, L.H., Aldubayan, S.H., Berry, G.T., Levy, H.L., 2018. Acute illness protocol for maple syrup urine disease. Pediatr. Emerg. Care 34, 64–67.

Schubert, C., van Langeveld, M.C., Donoso, L.A., 2014. Innovations in 3D printing: a 3D overview from optics to organs. British J. Ophthalmol. 98, 159.

Stegemann, S., Ternik, R.L., Onder, G., Khan, M.A., van Riet-Nales, D.A., 2016. Defining patient centric pharmaceutical drug product design. Aaps J. 18, 1047–1055.

Trenfield, S.J., Awad, A., Goyanes, A., Gaisford, S., Basit, A.W., 2018. 3D printing pharmaceuticals: drug development to frontline care. Trends Pharmacol. Sci. 39, 440–451.

Trenfield, S.J., Xian Tan, H., Awad, A., Buanz, A., Gaisford, S., Basit, A.W., Goyanes, A., 2019. Track-and-trace: novel anti-counterfeit measures for 3D printed personalized drug products using smart material inks. Int. J. Pharm. 567, 118443.

Vithani, K., Goyanes, A., Jannin, V., Basit, A.W., Gaisford, S., Boyd, B.J., 2019. An overview of 3D printing technologies for soft materials and potential opportunities for lipid-based drug delivery systems. Pharm. Res. 36, 4.

Vitoria, I., Merinero, B., Sánchez-Valverde, F., Gil, D., Dalmau, L., 2018. Enfermedad de orina de jarabe de Arce. In: Gil, D. (Ed.), Protocolos de diagnóstico y tratamiento de los Errores Congénitos del Metabolismo, 2 ed. Ergón, Madrid, pp. 85–94.

Wen, X., Gao, S., Feng, J., Li, S., Gao, R., Zhang, G., 2018. Chest-wall reconstruction with a customized titanium-alloy prosthesis fabricated by 3D printing and rapid prototyping. J. Cardiothoracic Surgery 13, 4.

Zema, L., Melocchi, A., Maroni, A., Gazzaniga, A., 2017. Three-dimensional printing of medicinal products and the challenge of personalized therapy. J. Pharm. Sci. 106, 1697–1705.

Zopf, D.A., Hollister, S.J., Nelson, M.E., Ohye, R.G., Green, G.E., 2013. Bioresorbable airway splint created with a three-dimensional printer. New England J. Med. 368, 2043–2045.