Development and evaluation of a 3D printing protocol to produce zolpidem-containing printlets, as compounding preparation, by the pressurized-assisted microsyringes technique

Nicolas Callede[a,b], Tommaso Masciotti[c], Luca Casettari[c], Nathalie Loosveldt[d], Jonathan Goole[a]

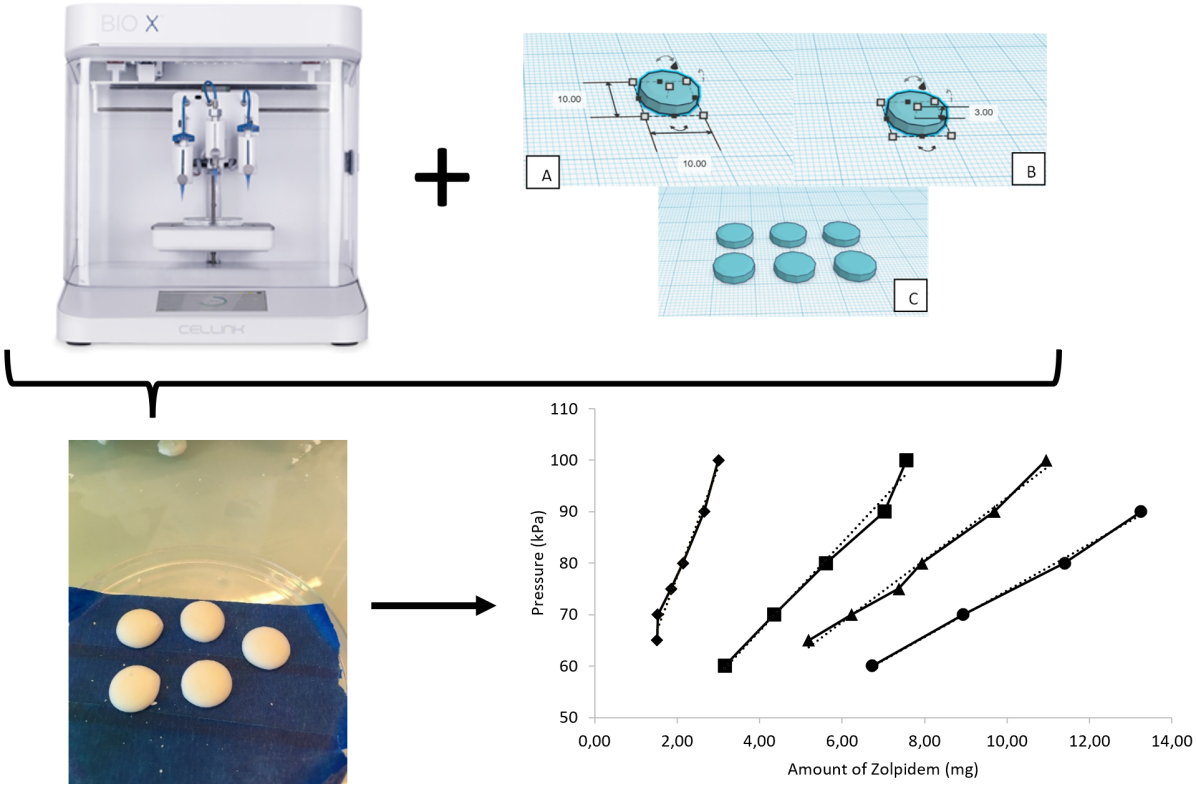
[a)] Laboratory of Pharmaceutics and Biopharmaceutics, Université libre de Bruxelles, Campus de la Plaine, CP207, Boulevard du Triomphe, Brussels 1050, Belgium

[b)] Lloyds Pharma Group, Avenue Pasteur 2, 1300 Wavre,Belgium

[c)] Department of Biomolecular Sciences, School of Pharmacy, University of Urbino Carlo Bo, Piazza el Rinascimento n°06, 61029 Urbino (PU), Italy

[d)] McKesson Company, SA Pharma Belgium-Belmedis NV, Avenue Emile Vanderveldelaan 82, 1200 Brussels, Belgium

Keywords: 3D Printing/PAM/Zolpidem/Compounding preparation/printlets



Introduction

Insomnia is a chronic disorder with a mean prevalence ranged from 6% to 15% worldwide, with strong variability between men (14%) and women (24%) [Khachatryan, 2021]. It is recognized as a global health problem since an early cross-sectional survey has outlined that almost 25% of adults reported poor sleep around the world, with 23.2% in the United States, 30% in France and 45.4% in China [Touitou, 2007; Xiang et al., 2021]. Methods of treating insomnia disorder include nonpharmacologic options such as stimulus control therapy, relaxation training, sleep restriction therapy, sleep hygiene, paradoxical intention therapy [Siebern et al., 2012]. The usual pharmacologic treatment for insomnia has been benzodiazepines and barbiturates. More recently, z-drugs (e.g., Zaleplon, Zolpidem and Eszopiclone) were introduced in the therapeutic arsenal to maximize benefits and minimize treatment damage [Poyares et al., 2005].

Zolpidem tartrate (N,N,6-trimethyl-2[4-methyl-phenyl]imidazole[1,2-a]pyridine-3-acetamide hemitartrate) is a short-acting ($T^{1/2}$ of 2.6h) non-benzodiazepine sedative-hypnotic drug whose primary indication is for sleep initiation problems [Monti et al., 2017]. The recommended initial dose is 5.0 mg for women as well as elderly patients (< 65 years-old) and 10.0 mg for non-elderly men [Orimo et al., 2006; Perlis et al., 2021].

A recent meta-analysis of randomized trial conducted by Xiang and co-workers has shown that there was no significant difference in the occurrence of adverse events (e.g., headaches, tolerance, rebound insomnia) after one month of Zolpidem and placebo treatment, regardless of a non-elderly or elderly subgroup [Xiang et al., 2021]. However, it was clearly described that their use must be limited to maximum four weeks. Indeed, it was demonstrated that long-term treatment (more than 12 weeks) with Zolpidem is strongly discouraged for patients suffering from underlying mental disorders, neurological diseases, medical conditions, or who have a history of substance or medication abuse [Monti et al., 2017]. Moreover, psychologic issues such as rebound anxiety and daytime agitation were also reported to occur with extended-duration administration of z-drugs [Scharf et al., 1994]. Such concern is essential as sedative medications have potential for misuse and abuse by patients [Weaver M.F., 2015]. For instance, in the United-States, prescription for Zolpidem increased five times more than the incidence of diagnosed cases of insomnia [Norman et al., 2017]. Such overuse is particularly hazardous for elderly as they are more susceptible to the negative consequences of Zolpidem use due to their lower clearance rates and higher maximum serum concentration [Glass et al., 2005]. It is obvious that prescription of these drugs in older patients is delicate, as ageing and comorbidity are usually associated with polypharmacy, where elderly take multiple medicines for long periods of their lives [Estrela M. et al., 2020]. This concern is all the truer as zolpidem seems to influence the evolution of the state and / or pathology of polymedicated patients. For instance, a nationwide population-based study performed in Taiwan on 101 719 patients, concluded that long-term treatment based on Zolpidem significantly increased the risk of Parkinson's disease as well as the unfavorable evolution of

it [Yang et al., 2014]. Studies have demonstrated that sleep disorders are common on chronic dialysis patients [Unruh et al., 2003]. Winkelmayer and co-corkers has concluded that, regardless of benzodiazepine or Zolpidem use, dialysis patients were at an increased risk of hip fractures [Winkelmayer et al., 2007]. Zolpidem also seemed to be contraindicated in patients suffering from gastroesophageal reflux as it increased the duration of esophageal acid reflux event [Gagliardi et al., 2009]. Moreover, Zolpidem was found to increase the risk of mortal infection due to higher risk of apnea episode and greater oxygen desaturation compared to placebo [Kripke et al., 2012].

Therefore, the dose of Zolpidem should be adjusted according to the gender, age, condition of the patient and the presence of polypharmacy. A first attempt was to develop 10.0 mg cutting tablets to be able to easily halve the dose for women and older patients [Monti et al., 2017]. However, it has been demonstrated that such tablets cannot be cut with sufficient accuracy of dose, even when a commercially tablets cutter is used. Indeed, research suggest that the variability may range from 50% to 150% of the targeted dose [Mohiuddin, 2019]. Faced with the therapeutic limitations inherent to marketed products, magistral preparations offer medical and legal alternatives to mass treatment. Indeed, patient-centered precision medicines may help to curb the inconveniences and drawbacks associated with mass treatments, the main one being the initiation of additional therapies, secondary to the appearance of adverse effects to primary treatments (e.g., use of antiparkinsonian drugs, antacids, antibiotics, anxiolytics). Therefore, compounding medicine is defined as the preparation of a therapeutic product for individual patient in response to an identified need [Pharmacy Board of Australia, 2015]. At the request of a prescriber, it offers the opportunity to customized dosage strength on small scale, wherever is in community and hospital pharmacies. However, pharmacies are exempt from GMP regulations. As a result, there is less assurance of consistent quality for compounded preparation than there is for FDA-approved drugs [Gudeman et al., 2013]. For instance, between 1990 and 2006, several studies conducted by the FDA has shown that 33% of compounding preparations failed to quality testing. Most of the failure were related to poor content uniformity, ranging from 59 % to 89 % [Gudeman et al., 2013; Mohiuddin, 2019]. Such issue is all the greater in the case of complex compounding (up to 268%), involving micro-doses (<25 mg) of active ingredients (e.g., Zolpidem) [Falconer and Steadman, 2017].

The main sources of dosage error in compound preparations having been identified as being associated with errors of dose calculations or measurement, the use of incorrect formulae, physical stability or wrong method of compounding [Minghetti et al., 2014], the use of a semi-automatic technique, with standardized protocol, should be valuable. In this way, 3D printing should be advantageously implemented as an alternative to standard compounding procedures.

3D printing techniques are based on digitally controlled deposit materials layer-by-layer to create freeform geometry. Among the different technologies available to create personalized dosage forms, it seems that most of them are not suitable to be used for compounding preparations [Goole

and Amighi, 2016]. Indeed, the drop-on-powder deposition technique is more designed to produce high drug-loaded dosage forms without the flexibility to easily modulate the dose [Zhou et al., 2014]. Fused-deposition modeling (FDM) is based on the use of a drug-loaded filaments which is a semi-finished product that does not correspond to the definition of raw materials used in compounding preparations [Henry et al., 2021]. Moreover, except through intense development, it is restricted to the use of thermostable drugs [Korpela et al., 2012]. Stereolithography is based on the use of polymerizable resin, the modulation of which in terms of formulation requires skills that go well beyond the know-how of a compounding pharmacist [Melchels et al., 2010]. Therefore, the pressure-assisted microsyringes (PAM) method was selected as it allows the tridimensional printing, and so the customization of the dose, by easily extruding a viscous semi-liquid material, called "slurry", through a syringe at room temperature [Chia and Wu, 2015]. The slurry being made from raw powders, the aim of this study will be to provide standardized protocol to the pharmacists about the mixing, the preparation, and the printing of the slurry. Zolpidem will be used as a model drug due to its evident advantage in personalized medicine.

Material & methods

Materials

All the excipients used in this work were of pharmaceutical grade. Lactose 450 mesh (DMV International®, Netherlands) was used as diluent; Microcrystalline cellulose (Avicel® PH101, FMC International, USA) was used as binder; Croscarmellose sodium (Ac-Di-Sol®, FMC, USA) was used as superdisintegrant; Hypromellose (Methocel® E15, Colorcon, France) was used as hydrophilic agent. Zolpidem (Fagron, USA) was used as a model drug due to its potential of use in personalized medicine.

Particles size distribution

The particle size distributions were obtained by laser diffraction using the Aero S dry powder dispersion system (Mastersizer® 3000, Malvern Instruments, Malvern, UK). Approximately 50 mg of powder were deposited on the tray of the Aero S dry dispersion plate and the vibration rate was fixed at 100%. The powder was dispersed at a shear pressure of 4 bars and the refractive index was fixed at 1.47, 1.53, 1.60 for cellulose derivatives, lactose, and Zolpidem, respectively. Size distributions were reported and characterized using the median volume diameter "Dv50".

Preparation of the slurry

The composition of the slurry allowed its extrusion through the syringes that were used during the 3D printing process (**Table 1**). 20 g of powders were manually mixed for 5 minutes using a mortar and a pestle. Additional experiments were performed on a Turbula® mixer Type T2C (WAB, Switzerland) and a planetary mixer KM80 (Kenwood, UK) to evaluate the efficacy and the feasibility of these methods in

a compounding pharmacy. In those cases, 100 g of powder mixture were used due to the volume constraint required by both methods. Then, the adequate amount of water was poured into the mixture to get the slurry. After homogenization, it was transferred into a 3 mL syringe which was especially designed for the 3D printer.

Table 1: Quantitative composition of Zolpidem-loaded printlets

Material	Composition (w/w %)
Zolpidem	2.60
Lactose 450 mesh	34.60
Avicel® PH101	13.60
Methocel® E15	2.20
Croscarmellose Na	1.00
Water	46.00
TOTAL	**100.00**

Quantification was done after each step of mixing to evaluate the homogeneity of the drug in the mixtures.

Design of printlets

Tinkercad™ was used as the computer aided design (CAD) program (**Figure 1**). It is a free, online 3D modeling program that runs in a web browser known for its simplicity and ease of use. Since it became available in 2011, it has become a popular platform for creating models for 3D printing as well as an entry-level introduction to constructive solid geometry in schools.

Tinkercad™ uses a simplified constructive solid geometry method of constructing models. A design is made up of primitive shapes that are either "solid" or "hole". Combining solids and holes together, new shapes can be created, which in turn can be assigned the property of solid or hole. In addition to the standard library of primitive shapes, the user can create custom shape generators using a built-in JavaScript editor.

Tinkercad™ exports models in STL or OBJ formats, ready for 3D printing.

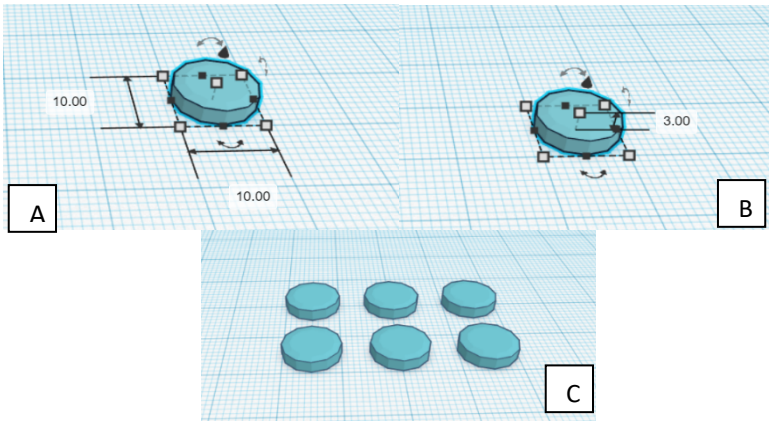


Figure 1: Printlet designed with Tinkercad™. A and B described the diameter and the height of the printlet, respectively. C represents a sample of 6 printlets

The standard cylindrical shape was used to develop the printlets because only this shape made it possible to modulate only 2 parameters (e.g., height and diameter) to assess the influence of the design on the adaptation of the final dose of Zolpidem in the printlets.

3D printing

Pressure-assisted microsyringes 3D printing was performed by the BIO X bioprinter from Cellink (USA) **(Figure 2)**.

Figure 2: BIO X bioprinter from Cellink (USA)

The temperature of the print bed may be controlled from 4°C to 60°C. The mixtures were extruded to be 3D printed on standard petri dishes. The software was Heart OSTM integrated system which may support .STL as well as Gcode file types.

Standard Pneumatic Printhead was used with standard 3 mL syringes which could be heated from 30°C to 60°C ± 0.5 C. The free flow plastic dispense tips were characterized by a diameter from 18G to 25G and a height of 32mm Cellink (USA) (**Table 2**).

Table 2: Characteristics of the free flow plastic dispense tips provided by Cellink as they are referred in the default parameters of the 3D printer

Color	Gauge	Inner diameter (mm)
Green	18	0.84
Pink	20	0.58
Purple	21	0.51
Blue	22	0.41
Orange	23	0.33
Red	25	0.25

The pneumatic pressure and the printing speed were ranged from 30 to 190 kPa and from 8 to 24 mm/s, respectively. The layer height was set from 0.1 mm to 0.6 mm. Six printlets were printed at once at each cycle of printing.

Post processing drying protocol

The printlets were immediately dried after printing in a Venti-Line convection drying oven (VWR, USA) at 40°C for 6 hours.

Thermogravimetric analysis

The residual water present in the printlets was assessed by thermogravimetric analysis (TGA) on a TGA Q500 (TA Instrument, UK). Samples of 5-10 mg were loaded into a platinum pan and were heated from 30°C to 150°C with a heating rate of 5°C/min under nitrogen gas (flow rate: 60ml/min). The percentage weight loss was recorded as a function of the temperature in triplicate (n=3).

Quantification

To extract Zolpidem from printlets, they were dispersed in HCl 0,1N under vortex until complete solubilization. Then, the solution was sonicated for 10min to be finally filtered with PES 0,45µm filter. Quantification was carried out spectrophotometrically with a Nanophotometer® NP80 (Implen®, Germany) at a wavelength of 295 nm.

Hardness, friability & disintegration

The hardness of the printlets was evaluated on a Kraemer EL Ektronik Tablet Hardness Tester (Darmstadt, Germany) (n=10). Friability evaluation was performed on an Erweka abrasion tester for 5 minutes at 100 rpm (Erweka, Langen, Germany) (n=10). Disintegration essays were done on a Sotax DT3 (Basel, Switzerland) (n=6).

Dissolution tests

A Distek 2100C USP 29 dissolution apparatus (Distek Inc., North Brunswick, NJ, USA), according to type I (basket) method, was used for the dissolution test (37.0 ± 0.2°C; 100 rpm). Release testing was carried out in 500 mL of HCl 0.01 M (pH 2.0). After 45 minutes, 10 mL of sample were withdrawn. The amount of Zolpidem released was detected spectrophotometrically with a Nanophotometer® NP80 (Implen®, Germany) at a wavelength of 295 nm. The percentages of drug release were measured at preselected time intervals and averaged (n=3).

Stability studies

Thanks to the USP guidelines for magistral preparations, compounding preparations must be stable for at least one month at ambient temperature [USP, Edition 42]. The stability of Zolpidem in the printlets was assessed for four weeks at 20 ± 4°C. The printlets were in a traditional pillbox, containing silica and stored away from sunlight (n=6).

Results and discussion

Preparation of the slurry

Compounding preparations are made from raw products, directly by the pharmacist, in a pharmacy open to the public or in a hospital. The 3D printing method using the PAM technique was selected in this work because it meets this criterion. To achieve it by 3D printing, the pharmacist must first make his slurry so that it can be easily extruded by the 3D printer. The entire preparation process should be reproducible, robust, and relatively simple. The importance of producing stable mixtures to ensure the uniformity of dosage units with respect to the active ingredient, particularly low-dose potent active ingredients, cannot be overstated.

Indeed, Zolpidem was present in a low dose in the mixture and the median diameter of the particles was 34.4 µm. Several solutions can be envisaged to include a small amount of micronized powder in a mixture. The homogeneity has been shown to be more dependent on the size distribution of the other components than on the shape of the particles. Consequently, the use of excipients having an average diameter greater than 50 µm is usually preferred to avoid the presence and persistence of potential agglomerates [Swaminathan and Kildsig, 2002]. However, the production of an extrudable slurry calls for the same requirements as a granulation process to bond the particles together and to avoid demixing during the process. Therefore, 450 mesh lactose was selected (d0.5 = 27 µm). In order to allow the printlets disintegrating quickly and releasing 80% of Zolpidem within 45 minutes, Methocel® E15 (d0.5 = 83µm) and croscarmellose sodium (d0.5 = 40 µm) were added to increase hydrophilicity and the disintegration capacity of the printlets, respectively. Avicel® PH101 (d0.5= 48µm) was used as the binder. Then, 3 mixing techniques, easily implantable in a compounding pharmacy, were evaluated: manual mixing with mortar and pestle; Turbula® and the use of a planetary mixer. It has

previously been shown that in mixtures of micronized drug and carrier, the pattern of change in the coefficient variation with mixing time was attributed to the following sequence — distribution of agglomerates of micronized drug during convective mixing; breakdown of agglomerates during shear mixing; and the distribution of the primary particles during the diffusive mixing stage. Consequently, when the shear forces are sufficiently high, it is possible to homogenize small quantities of powder (e.g., Zolpidem) in a mixture of excipients characterized by an average particle diameter lower than 50 µm [Swaminathan and Kildsig, 2002].

The quantification of Zolpidem was carried out on the mixture of dry powders and in the slurry, at 5 different places (n=5). An average of the percentage deviation from the theoretical value was calculated (**Table 3**).

Table 3: Mean percentages of deviation between the theoretical and experimental amount of Zolpidem in the mixture of dry powders and in the slurry (n=5)

	Turbula® (%)	**Planetary Mixer (%)**	**Mortar & pestle (%)**
Powders	1.10 ± 0.22	1.80 ± 0.41	1.12 ± 0.28
Slurry	1.30 ± 1.00	1.16 ± 0.07	1.04 ± 0.03

As it can be observed in **Table 3**, the three evaluated mixing methods provided deviations lower than 2% (w/w). This demonstrated that Zolpidem was homogeneous, both in the powder and in the slurry, before the start of printing. It can also be observed that the pharmacist can, as in any standard compounding preparation, carry out his own mixing manually. Therefore, for the rest of this work, all the mixtures were carried out using a mortar and pestle prior to printing.

Post-processing drying protocol

TGA were performed on the mixture of the raw excipients to evaluate their residual moisture percentage. Before the experiment, the excipients were stored at room temperature as they should be in conventional compounding pharmacy. It was shown that the dried excipients themselves presented a percentage of residual moisture of 4.30 ± 0.02% (n=9) before the addition of water to make the slurry.

The PAM technique being a 3D printing process based on the use of solvent (e.g., water), a post-treatment is necessary to eliminate the residues. Indeed, in the case of water, excessive humidity could increase the risk of microbial contamination. Therefore, before any development involving this 3D printing technique, it is necessary to set the temperature and the drying time to remove the residual solvents from the printlets.

Theoretically, the higher the drying temperature, the shorter the time required to remove the solvents.

It was observed that beyond 40°C (e.g., 50 & 60°C), the mass of the raw mixture of the excipients decreased by more than 2% w/w after 4 hours of drying. Consequently, it was decided to set the drying temperature at 40°C to properly manage the speed of drying and the stability of the material. In compounding pharmacies, lab-scale ventilated ovens can be easily acquired at low cost.

The residual moisture of the printlets was evaluated by TGA (**Figure 3**).

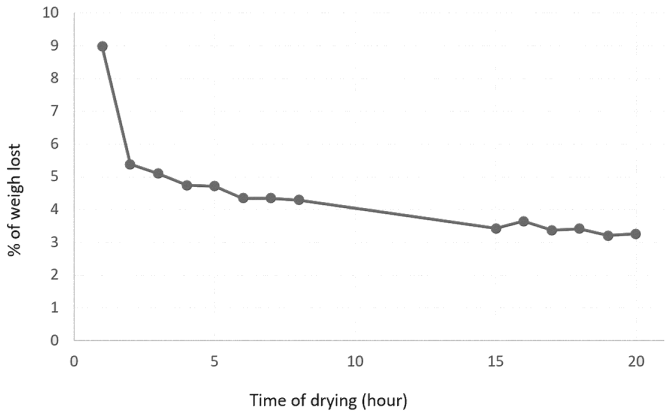


Figure 3: Profile of weigh loss of the printlets when placed in an oven at 37°C

As it can be seen, the percentage of weight loss reached 4.30 % (w/w) after 6 hours. Then, a plateau was observed. In order to not increase the time of drying, which allow the deliverance of the compounding preparation within a day, it was decided to fix the time of drying at 6 hours after printing.

3D printing

Selection of the printer

The BIO X bioprinter was initially designed to be used in tissue engineering, drug discovery, toxicity research, and enabling researchers to do more in 3D cell culture faster. This type of printer was selected because its extrusion system is based on pneumatic dispensing system.

Compared to a pneumatic dispensing system where slurry extrusion is delayed due to the compressed volume of the air, a mechanical dispensing system offers better control of the deposition through direct extrusion of the material. In addition, systems based on the use of an endless screw allow the printing of slurry of higher viscosity and therefore offer a wider range of potentially extrudable material [Murphy, 2014]. Despite the apparent disadvantages of using a printer based on a pneumatic dispensing system, it was decided to use the Bio-X because its simpler drive-mechanic component allows easier use and maintenance (e.g., cleaning). Indeed, whatever the pharmaceutical manufacturing process envisaged, it is essential to consider both the precision, robustness, and reproducibility of the method but also the ease of maintenance. It seemed obvious that this last criterion was all the truer for a compounding pharmacy application. Therefore, the use of a printer with a pneumatic dispensing system has been preferred to other types of printers. In addition, as it will be shown, the technology used did not reduce the expected performance from the printing.

Design of the printlets

It was previously demonstrated that it was possible to modulate the dose of a drug within a dosage form, from the same formulation, by only modulating its volume by 3D printing [Pietrzak et al., 2015]. Therefore, one of the first choices that should be made was to select the appropriate shape of the printlets. The easiest would have been to print spheres because only one parameter could have been modified (e.g., diameter) to modulate the amount of Zolpidem inside the printlets. However, the PAM technique does not allow producing this type of shape because a sufficiently wide base onto the build platform is essential to avoid the spreading of the slurry during printing. The cylindrical shape was preferred to an oblong shape because only its diameter and its height determine its final volume. In order not to reduce the patient's compliance due to too large printlets, a diameter of 1 cm was arbitrarily fixed to only must assess the influence of their height on the final dose of drug.

However, each technique and each printer have their own characteristics which may influence the production methodology. For instance, the model of 3D printer used in this work can only change the height of the printed layers depending on the internal diameter of the tips used during the printing. Consequently, when designing the dosage form in Tinkercad™, it was essential to fix the height of the model in such a way that it was possible to achieve an integer number of layers. Otherwise, it would have been impossible to properly control the volume and, therefore, the dose of Zolpidem inside the printlets. Indeed, a 20 gauges tip has an internal diameter of 0.058 cm (**Table 2**). If 3 or 4 layers must be printed to assess their influence on the final dose of drug inside the printlets, it was necessary to set the height of the dosage form at 0.174 cm (3x0.058 cm) and 0.232 cm (4x0.058 cm), respectively. Indeed, if the height of the model had been set at 0.200 cm, then the number of layers would have been 3.45 (0.200 x 0.058 cm). As the printer could not print a layer subunit, the printlets would have only counted 3 layers which leads to an error of 15% only due to an inadequate design. Moreover, while the height of the printlets would have been increased from 0.174 cm (e.g., 3 layers) to 0.200 mm (e.g., 3.45 layers), no notable difference would have been observed on both batches of printlets.

Printability

As already explained, one of the fundamental parameters of printing was to determine the type of tips to use according to their internal diameter and the ability of printing.

By using tips with gauges lower than 20, the printlets produced presented imperfections due to a collapse of the form during printing (**Figure 4**). It was clearly visualized that this phenomenon was due to too much dispensing of slurry during printing. Indeed, with this printer model, even by reducing the pressure, it was not possible to reduce the extrusion speed and therefore the extruded volume.

(A)  (B)  (C) 

Figure 4: Printlets obtained from (A) 18G; (B) 20G and (C) 22G (Pressure=80 kPa; Speed = 15mm/s; Grid= Rectilinear; Infill density= 100%)

In contrast, a diameter lower than 0.041 cm (e.g., 22G) did not allow the extrusion of the slurry within the pressure limits proposed by the printer. Therefore, preliminary tests were carried out with 20 and 22G tips. As can be seen in **Figure 4**, the resolution of the printlets (lower spreading of the slurry) was visually higher from the 20G tips which were used for the rest of the work.

Once the design and the choice of tips to use were fixed, only a few parameters could still be modulated in the 3D printer. These were technical parameters which could be considered as difficult to assimilate and control by a compounding pharmacist. Therefore, it was not found judicious to evaluate their potential influence on the modulation of the desired dose of Zolpidem in the printlets. Because after several unsuccessful trials, no objective relationship between the viscosity of the slurry and the ability of its impression was demonstrated, a trial-and-error-based methodology was applied to determine the pressure, the speed and the microarchitecture of printing which was needed to allow the printability of our slurry using 20G tips.

The aim was to propose a model where a minimum of parameters should be modulated to obtain the desired dose of drug. By proposing this goal, the following printing parameters were initially applied to evaluate the influence of the volume (e.g., number of layers), of the mass of dry printlets and of the infill density on the modulation of the amount of Zolpidem inside the printlets. The pressure and the speed of printing were set at 80 kPa and 15mm/s, respectively. The microarchitecture of the printlets was based on rectilinear grid.

Influence of the volume and dried mass of the printlets

As the diameter of the cylinder was fixed, its volume only depended on the number of printed layers (**Equation 1**). Depending on the number of layers, the volume of the cylinder was automatically calculated in Tinkercad™.

Equation 1 : $V = \pi r^2 h$

V: volume of the cylinder

r = radius of the cylinder

h= height of the cylinder

Therefore, five different models were evaluated to assess whether it was possible to modulate the amount of Zolpidem in the printlets according to their volume or their dried mass (**Table 4**).

Table 4: Amount of Zolpidem according to the number of layers, the volume, and the dry mass of the printlets

Number of layers	Volume (cm^3)	Dry mass (mg)	Amount of Zolpidem (mg)
3	0.14	79.66	3.97
4	0.18	89.40	4.37
5	0.23	116.55	5.94
6	0.27	162.53	8.15
7	0.32	181.30	9.06

As it can be observed in **Figure 5**, the relationship between the amount of Zolpidem in the printlets and their theoretical volume was not linear ($R^2 = 0.9587$). In contrast, the amount of Zolpidem in the printlets linearly evolved with their dry mass ($R^2 = 0.999$).

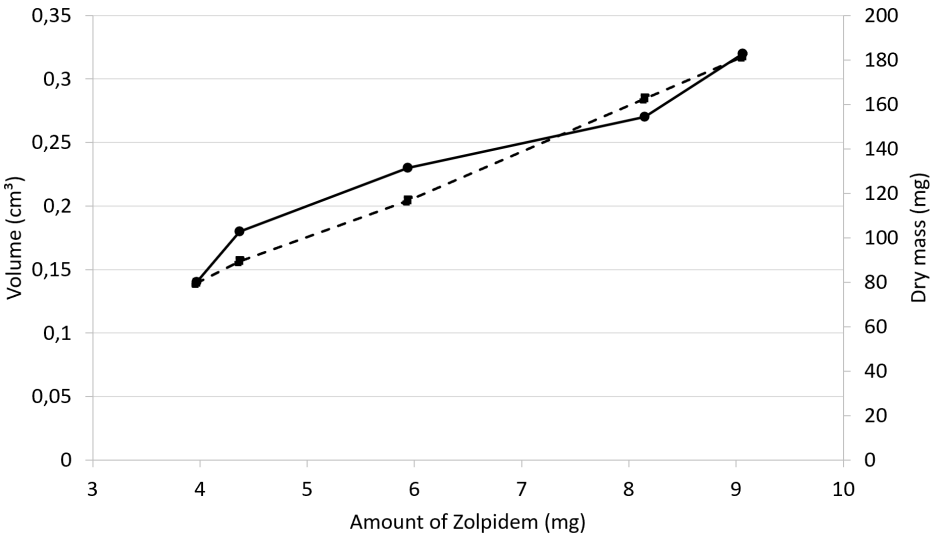


Figure 5: Amount of Zolpidem according to the volume (cm^3) (●) and dry mass (mg) (▪) of printlets when modulating the number of layers

These observations meant that the printer did not extrude an amount of slurry linearly proportional to the volume calculated by the drawing program. This was certainly due to the well-known effect of the die angle on the extrusion. Indeed, the final volume of an extruded form is usually greater than that initially evaluated, especially when the material is characterized by elastic properties such as cellulose derivatives (e.g. Avicel®, Methocel® and Croscarmellose) [Liang, 1995].
However, it was also shown that the amount of drug was homogeneously dispersed in the slurry and did not undergo any untangling during printing as it was proportional to the dry mass of the printlets.

Up to now, it was possible to conclude that the volume alone, which was associated with the number of layers, did not allow controlling the amount of Zolpidem in the printlets. It was necessary to deeper investigate the influence of the diameter and the infill on the capacity of our method to modulate the dose of drug in a controlled and anticipated manner. It was also interesting to assess whether the amount of Zolpidem also changed with the mass of wet printlets. In this case, this parameter, which could be evaluated immediately after printing, could be used as quality control parameter by the pharmacists.

Influence of the diameter

In order to evaluate the influence of the diameter on the ability of the method to properly modulate the amount of Zolpidem in the printlets, new models were designed. They were characterized by different diameters with a height fixed at 0.174 cm, which corresponded to 3 layers. According to **Equation 1**, Thinkercad™ automatically calculated the theoretical volume of the printlets (**Table 5 & Figure 6**).

Table 5: Amount of Zolpidem according to the diameters, the volume, and the dry mass of the printlets

Diameter (cm)	Volume (cm^3)	Dry mass (mg)	Amount of Zolpidem (mg)
0.8	0.09	45.95	2.36
0.9	0.11	48.00	2.40
1.0	0.14	62.19	3.07
1.1	0.16	75.67	3.69
1.2	0.20	97.96	4.84
1.3	0.23	128.24	6.31

The main difference with the previous study was that the diameter could be finely modulated during the design of the printlets which allowed a hypothetic better control of the amount of Zolpidem. Indeed, the number of printable layers was limited to an unfractionated number which drastically reduced to flexibility of modulating the dose of the drug in the printlets.
However, as it could be observed in **Figure 6**, the relationship between the amount of drug in the printlets and their theoretical volume was still not linear (R^2 = 0.9554). In contrast, the amount of

Zolpidem in the printlets linearly evolved with their dry mass (R^2 = 0.994). Other functions, such as logarithmic or exponential, were also evaluated to find a better relationship but none of them has shown a better correlation.

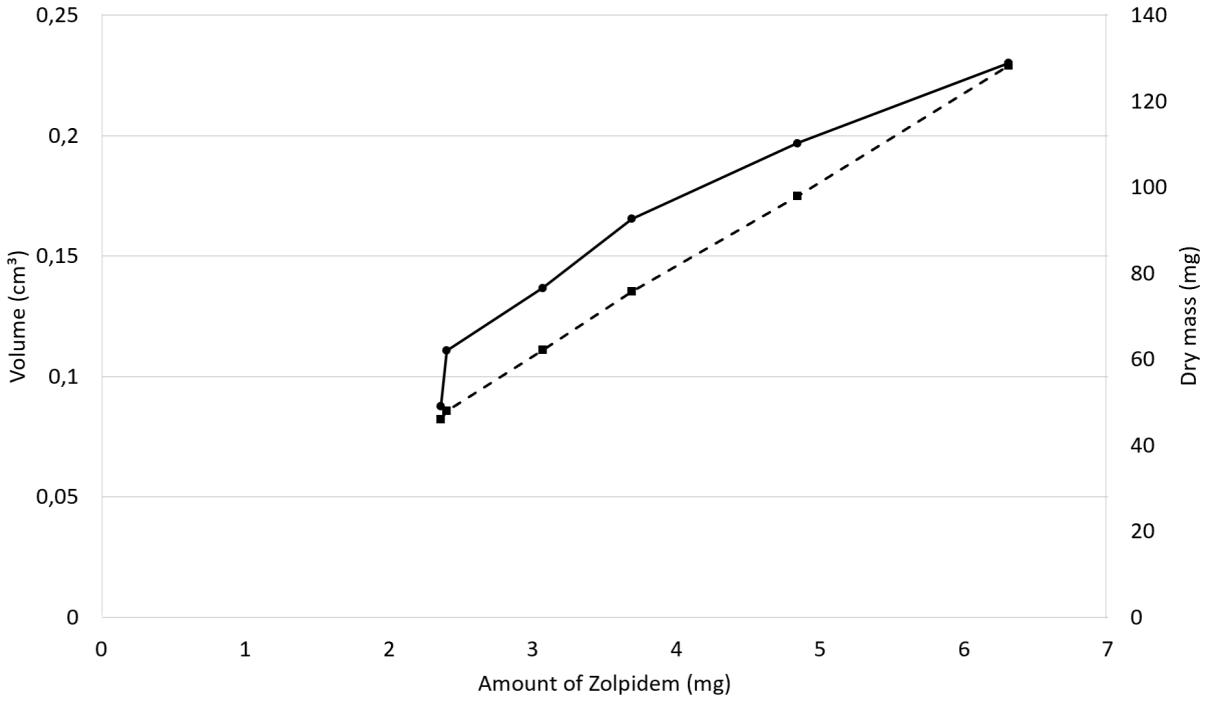


Figure 6: Amount of Zolpidem according to the volume (cm^3) (●)and dry mass (mg) (▪) of printlets when modulating their diameter

Nevertheless, it seemed that it was still due to a physical phenomenon inherent to the extrusion as, even by carrying out very small variations of diameter (max. 1 mm), the technique demonstrated its robustness since the amount of Zolpidem in the printlets has evolved linearly with their dry mass.

Therefore, it seemed that a single parameter directly impacting the theoretical volume of the printlets (e.g., height & diameter) did not allow obtaining a significant relationship with the amount of Zolpidem in the dosage forms. However, it was desirable because it would allow the pharmacist modulating the doses of the drug very easily according to the need of their patients.

It was decided to fix the diameter at 1 cm and the height of the printlets to evaluate the influence of the infill on the modulation of the amount of Zolpidem in the printlets.

Influence of the infill percentage

As stated by Mohanty et al., the infill density is the parameter that defines the amount of material filled into the object and subsequently relates to the porosity of the 3D printed structure [Mohanty et al., 2015]. The infill density can range from 0% to 100%, where 0% results in a completely hollow object

and 100% infill results in a completely solid object **(Figure 7)**. The infill pattern has also shown to be an important parameter to consider as it greatly influenced the strength of the 3D printed object [Abbas et al., 2017]. However, this importance has mainly been demonstrated in FDM 3D printing process and seems less critical in PAM. Therefore, a rectilinear pattern was used because it responded to the low resolution inherent this 3D printing technique [Chia and Wu, 2015].

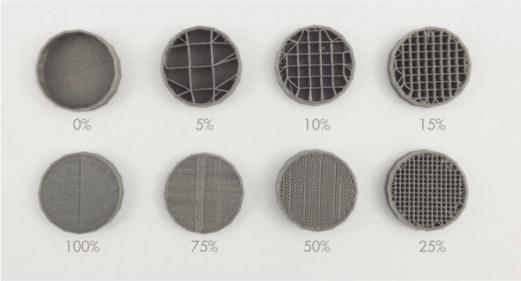


Figure 7: Examples of infill percentage from 0% to 100%

The influence of the infill percentage was evaluated from 2 heights of printlets, corresponding to 4 and 5 printing layers to better assess the relevance of the observed results. Under 40% of infill, it was visually observed that the resolution of the printlets was erratic during the printing. Indeed, the slurry was discontinuously extruded during the filling of the dosage forms.

Above 80% of infill, no more noticeable difference could be observed. The amount of Zolpidem in the printlets peaked from this percentage, demonstrating the limits of the PAM technique when fine resolutions are requested **(Figure 8)**.

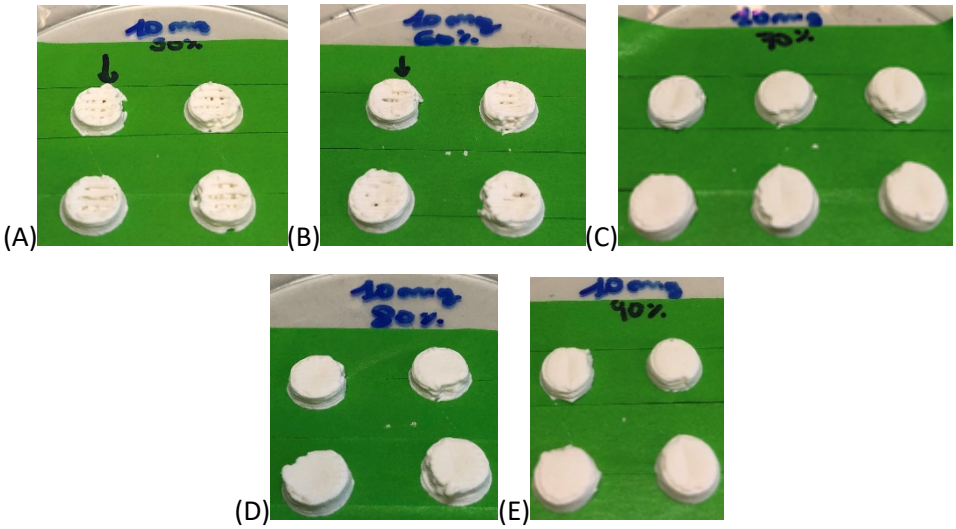


Figure 8: Zolpidem-loaded printlets with (A) 50%, (B) 60%, (C) 70%, (D) 80% and (E) 90% of infill (4 layers, 1 cm diameter)

At both cylinder heights studied, the amount of Zolpidem in the printlets linearly evolved with the percentage of infill, reaching correlation coefficients equal to 0.9957 and 0.9921, from 4 and 5 layers, respectively (**Figure 9**).

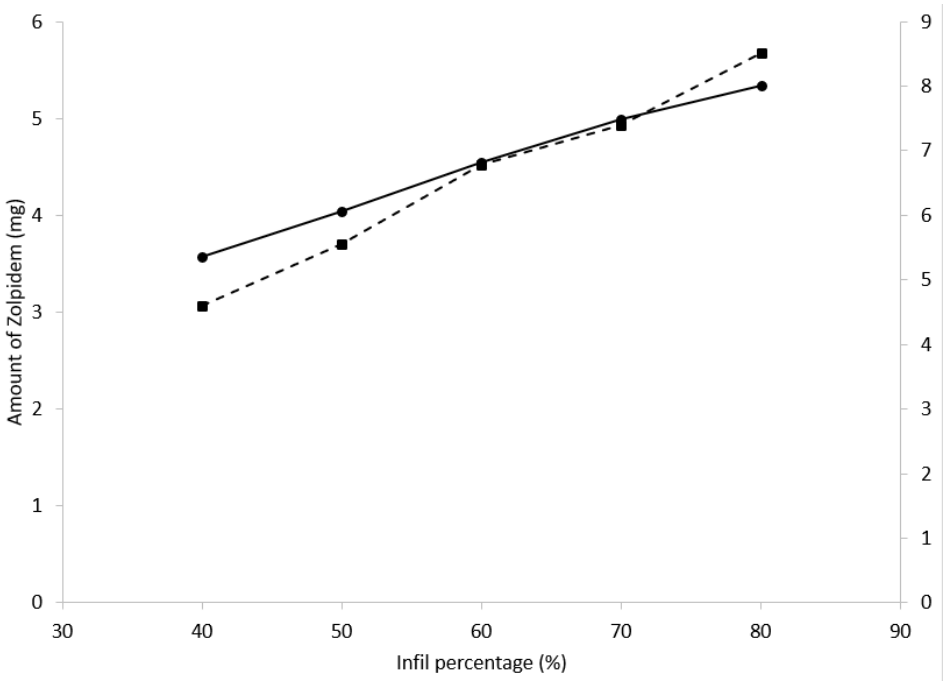


Figure 9: Amount of Zolpidem according to the infill percentage from 4 layers (●) and 5 layers (▪) printlets

However, due to the relative low resolution at percentages of infill outside the 40-80% range, it seemed difficult to finely modulate the amount of Zolpidem only by varying the percentage of infill. In addition, this linearity was obtained by varying the percentage every 10%. Modulating it individually would also present resolution concerns.

As previously observed, it seemed that the PAM 3D printing technique offered enough precision to be able to produce personalized medicine. However, modulating a single printlet design parameter (e.g., diameter, height, infill) did not seem to allow targeting a precise dose of Zolpidem inside the printlets. Indeed, due to the multi-factorial aspect of the technique, adapting the dose of drug required the simultaneous adaptation of several parameters.

Therefore, it was proposed to develop standard protocols for each desired dose of Zolpidem. They will be designated and encoded in the print file. Then, the pharmacist will simply have to select the right protocol. Such procedure corresponds to a magistral therapeutic form in which the pharmacist follows a previously validated procedure.

Development of the protocols

Determination of the dimension of the printlets

Zolpidem-containing immediate-release tablets are mainly dosed at 10 mg in the market.

Usually, to progressively reduce the dose of drug, prescribers recommend the tablets to be cut in half to obtain 5.0 mg of drug. However, it has previously been demonstrated that such a practice may generates a variability ranged from 50% to 150% of the targeted dose [Mohiuddin, 2019].

In addition, if the breakability of the tablets into 2 parts assumed to be identical in dose of drug may easily be envisaged, a finer adaptation, and more specifically, a more gradual reduction in the doses of Zolpidem is impossible to obtain without compounding preparation.

Therefore, it was decided to develop and validate printing protocols for printlets containing 10.0, 7.5, 5.0 and 2.5 mg of Zolpidem to offer the possibility for the patient to get a very progressive decrease of his daily intake of sedative.

By combining all the data previously obtained from the evaluation of the influence of the printlet's diameter and their number of layers to their volume, and therefore to the loaded amount of Zolpidem (**Tables 6 & Figure 10**), it was possible to get a correlation allowing to theoretically fix the height and diameter to get the adequate volume of printlets, and therefore to reach the targeted doses of drug (**Figure 10**). It was decided to set the percentage of infill at 70% because it generated the smallest deviation dose of Zolpidem according to the number of layers (**Figure 9**) and 60% did not offer visually homogeneous printlets (**Figure 8B**).

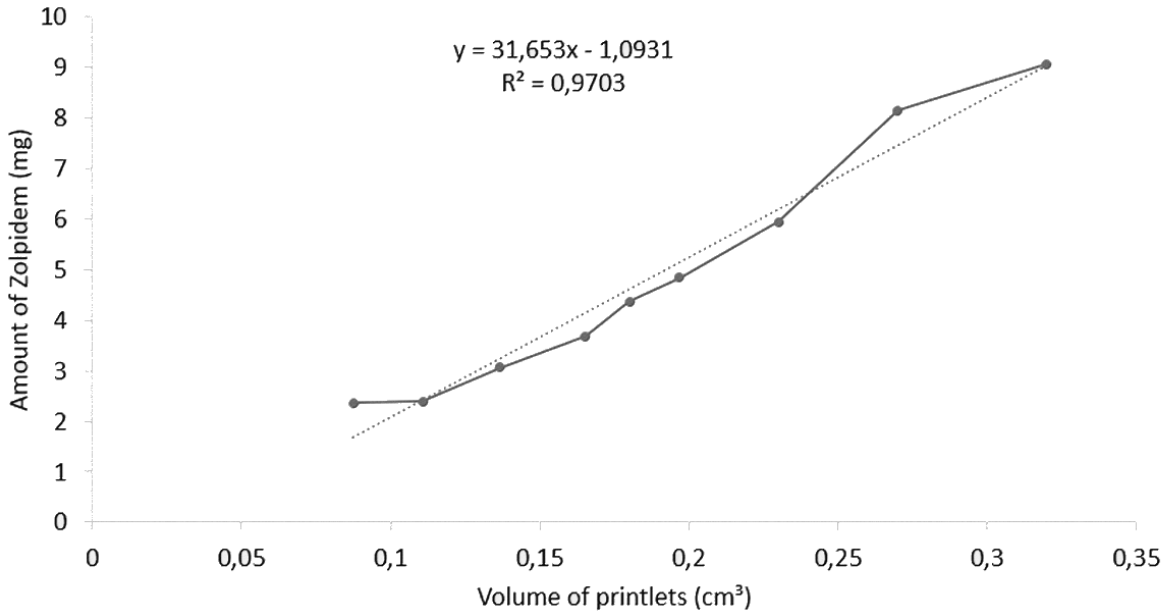


Figure 10: Relationship between the volume of printlets and the amount of Zolpidem using different diameters and numbers of layers

As modulating the height of the printlets could only be done as a function of the number of layers which must be a whole number, Tinkercad™ made it possible to finely modulate the diameter to obtain the desired volumes, while setting the number of layers to print (**Table 6**).

Table 6: Fixed dimensions of printlets to get 10.0, 7.5, 5.0 and 2.5 mg of Zolpidem

Amount of Zolpidem (mg)	Volume of printlets (cm^3)	Number of layers	Diameter (cm)	Height (cm)
10.0	0.350	6	1.13	0.348
7.5	0.271	6	0.99	0.348
5.0	0.192	4	0.84	0.232
2.5	0.113	3	0.64	0.174

In order to develop an efficient printing procedure for the pharmacist, it was necessary to set the volume of the printlets to obtain the desired dose of Zolpidem. The second step was to evaluate the printing parameters that would make it possible. Several optimized print settings have already been set such as the gauge of the tips, the speed of printing and the microarchitecture of the printlets which were fixed at 20G, 15 mm/s and based on rectilinear grid, respectively.

As previously observed, the printing pressure allowing the slurry to be extruded through the tips strongly influenced the amount of material that was deposited on the building platform, which greatly influenced the final dose of Zolpidem in the printlets. Therefore, this parameter has been specifically evaluated.

Determination of the pressure of printing

Several trials were performed at 60kPa; 70kPa; 80kPa; 90kPa and 100kPa from the previously fixed models **(Figure 11)**.

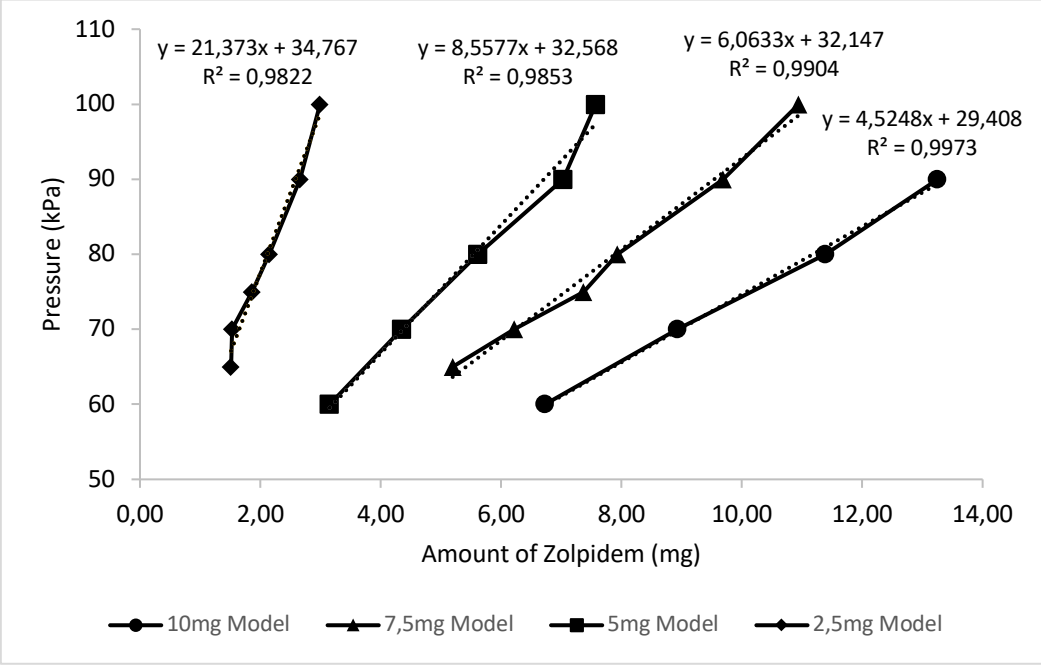


Figure 11: Relationships between the amount of Zolpidem (mg) in the printlets and the pressure of printing (kPa)

As it may be observed in **Figure 11**, the amount of Zolpidem linearly increased with an increase of the pressure of printing. Moreover, the slopes of the regression curves showed that small variations of it more strongly influenced printlets characterized by larger volumes. It meant that, compared to smaller models, a similar variation of pressure lead to a higher variation of Zolpidem's content in larger models. At similar printing speed, the printer took more time to print dosage forms with higher volume which generated more variation in the amount of extruded slurry for low pressure variations.

To perform quality control evaluations, the printing pressures were fixed at 88 kPa, 75 kPa, 77 kPa, 75 kPa from the 2.5 mg, 5.0 mg, 7.5 mg and 10.0 mg models, respectively.

Quality control

The dimensions of the printlets and the printing protocols having been fixed for each model, it was interesting to propose a simple and rapid method to extemporaneously evaluate whether the adequate dose of Zolpidem has been obtained, using a non-destructive method.

Correlation between dry mass and wet mass

Up to now, the amount of Zolpidem in the printlets has always been evaluated by grinding and dissolving the dried dosage forms. If this procedure could be easily performed during the development phase, the pharmacist must be able to directly assess the quality of the printlets in their wet form. Moreover, as spectrophotometric quantification is almost impracticable in a compounding pharmacy, a method of evaluation based on the uniformity of weight has been proposed.

It has already been observed that the mass of the wet printlets linearly evolved with that of the dry printlets (R^2 = 0.9975). As it was also characterized by a linear relationship (R^2 = 0.9974), it was also possible to assess the amount of Zolpidem by evaluating the mass of wet printlets (y = 0,0264x + 0,2047), which allow the pharmacist proceeding a quality control of his magistral preparation immediately after printing **(Table 7)**.

Table 7: Amount of Zolpidem (mg) in the dry printlets corresponding to the weight of wet printlets (mg) and percentage of deviation from the targeted doses (mg) (n=1)

Targeted dose of Zolpidem (mg)	Amount of Zolpidem in the dry printlets (mg)	Deviation (%)	Weight of wet printlets (mg)
2.5	2.66	+6.4%	92.25
5.0	5.35	+7.0%	200.47
7.5	7.37	-1.7%	264.75
10.0	9.00	-10%	336.25

Moreover, all printlets responded to the Zolpidem-containing tablets monograph of the US pharmacopoeia Edition 42 which mentioned that such tablets should contain at least 90.0% (w/w) and a maximum of 110.0% (w/w) of drug [USP, Edition 42]. However, as deviations from the targeted doses were observed, it seemed mandatory to evaluate the effect of inter-day variation on the accuracy of the method.

Accuracy of the method

According to ISO 5725-1, the general term accuracy is used to describe the closeness of a measurement (e.g., effective dose of Zolpidem in the printlets) to the true value (e.g., theorical targeted value of Zolpidem in the printlets) [ISO 5725-1, 1994]. Accuracy may be evaluated to assess the ability of the printing method to provide the desired dose of Zolpidem from different slurry preparations and printing sequences at different days, as it will be done in compounding pharmacy.

As the relationship between the amount of Zolpidem in the printlets and their wet mass was shown to be linear, 18 printlets of each model were printed to determine the tolerance range of their wet mass to obtain a dose Zolpidem that met to USP42 criteria (90-110%) (**Table 8**).

Table 8: Weight of wet printlets (mg) to be achieved to meet the tolerance range of Zolpidem (mg) that met to USP42 criteria (90-110%) for each model

Model (mg)	Range of acceptability of wet printlets (mg)	Tolerance range of Zolpidem that met to USP42 criteria (90-110%)
2.5	77.72 – 96.27	2.25 -2.75
5.0	161-19 – 198.28	4.50 – 5.5
7.5	244.65 – 300.29	6.75 – 8.25
10.0	328.11 – 402.30	9.00 – 11.00

If the wet mass of printlets was out of these ranges for each model, it was found that the amount of drug in the printlets did not response to the USP42 criteria of acceptability of Zolpidem-containing tablets. It was previously observed that deviations from the targeted doses may appear, probably due to the inherent variability of the rheological properties of the slurry as well as of the printer itself.

Therefore, depending on the experimenter or the temperature and humidity conditions when preparing the slurry, its extrusion may lead to the printing of wet printlets whose mass is outside the acceptance range. Thanks to this printing method, the pharmacist can directly ensure the quality of his printlets. To do this, a decision tree was designed (**Figure 12**).

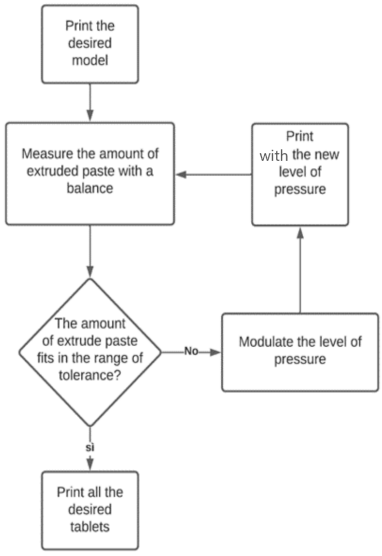


Figure 12: Decisional tree to ensure a quality control of Zolpidem-containing printlets

The pharmacist can print a single unit form according to the desired dose of Zolpidem in the printlets and, therefore, the selected model (2.5; 5.0, 7.5 or 10 mg). If the wet mass of this single printlet is within its tolerance range (**Table 8**), the pharmacist can launch a complete printing protocol of 6 printlets. If its wet mass is outside the tolerance range, the printing pressure may easily be increased or decreased to increase or decrease the wet mass of the printlet, respectively.

For instance, at Day 1, single printlet was printed using the 2.5 mg model at 88 kPa. Its weight after printing did not fit with the tolerance range (e.g., 65 mg instead of minimum 77.72 mg). The printing pressure was increased to 105kPa to get a wet printlet characterized by a weight of 85 mg which was within the range of tolerance. Then, a batch of 6 printlets at 105kPa was produced as the weight of the wet printlets was in the tolerance range (e.g., 87.83 mg) as well as the amount of Zolpidem in the printlets (**Table 9**). In contrast, at Day 2, single printlet was printed using the 7.5 mg model at 75kPa. Its weight after printing did not fit with the tolerance range (e.g., 321mg instead of maximum 300.29 mg). The printing pressure was decreased to 70kPa to get a wet printlet characterized by a weight of 273mg which was within the range of tolerance. Therefore, a batch of 6 printlets at 70kPa was produced as the weight of the wet printlets was in the tolerance range (e.g., 288.66 mg) as well as the amount of Zolpidem in the printlets (**Table 9**). By following this decision tree, 6 printlets were printed, for each model, at 2 different days (Day 1 & Day 2), with different slurries (**Table 9**).

Table 9: Weight of wet printlets and amount of Zolpidem in the printlets at 2 different days and slurries (n=6)

Day 1	2.5 mg model	5.0 mg model	7.5 mg model	10.0 mg model
Weight of wet printlets (mg)	87.83 ± 0.02	175.00 ± 1.02	279.50 ± 0.54	365.83 ± 0.73
Amount of Zolpidem (mg)	2.49 ± 0.06	5.17 ± 0.12	7.92 ± 0.47	9.45 ± 0.86
Day 2				
Weight of wet printlets (mg)	85.83 ± 0.05	175.66 ± 0.86	288.66 ± 1.32	373.33 ± 0.99
Amount of Zolpidem (mg)	2.49 ± 0.07	4.90 ± 0.13	7.88 ± 0.28	10.38 ± 0.42

The developed protocol allows printing 24 printlets (6 printlets x 4 models) with the same slurry. Moreover, using 2 different slurries, the decisional tree was shown to succeed in immediately providing reliable information on the quality control of the printlets. The pharmacist can extemporaneously modulate the printing pressure, on the printer itself, to get the targeted dose of Zolpidem.

Indeed, the accuracy of the method was found to be acceptable as all printlets responded to the Zolpidem-containing tablets monograph of the US pharmacopoeia Edition 42 which mentioned that such tablets should contain at least 90.0% (w/w) and a maximum of 110.0% (w/w) of drug [USP, Edition 42], regardless of the day of production.

Hardness, friability & disintegration

After the friability test, the average weight lost was found to be lower than 1.00% (w/w) as it only reached 0.67 ± 0.02% (w/w). Their hardness was 10.79 ± 0.13 N which meant that the variability was only 1.20%. Their disintegration time was lower than 15 minutes in HCl 0.1N.

Dissolution test

For slowly dissolving or poorly water-soluble drugs (e.g., Zolpidem), 85% (w/w) dissolution is recommended by the FDA after 30, 45 or 60 minutes to ensure the quality of the product [FDA, 1997]. It was shown that the printlets responded to this recommendation as the entire amount of Zolpidem was released from them after 45 minutes in 500 mL of HCl 0.01 M, regardless of its dose in the printlets (pH 2.0; 37.0 ± 0.2°C; basket method; 100 rpm; n=3)

Stability studies

The stability study was carried out on printlets containing 10 mg of Zolpidem (n=6).

The percentage of the drug in the printlets reached 100.2 ± 0.07, 99.93 ± 0.03, 99.95 ± 0.04 and 99.33 ± 0.3 % (w/w) after one, 2, 3 and 4 weeks, respectively. Therefore, it could be concluded that the Zolpidem-loaded printlets responded to the USP guidelines for magistral preparations which

mentioned that compounding preparations must be stable for at least one month at ambient temperature [USP, Edition 42].

Conclusion

A printing technique, based on the use of pressurized-assisted microsyringes method, has been developed. The methodology, as well as the protocols, met the current definition of a compounding preparation and can therefore be used, within the legal framework, by the compounding pharmacist. In contrast to the other 3D printing methods, including that based on fused-deposition modeling, the PAM technique avoids cross-contamination because each syringe only contains one formulation. It can, unlike a printing nozzle, be disposable. Moreover, the developed protocols demonstrated the accuracy of the method.

Using Zolpidem as a model of drug, a decision tree was developed for four models. It allowed the compounding pharmacist carrying out a quality control on the printlets immediately after printing.

It has been demonstrated that this methodology made it possible to obtain printlets that responded to the Zolpidem-containing tablets monograph of the US pharmacopoeia Edition 42. It is essential to remember that these standards have been enacted for tablets industrially produced. The compounding preparations proposed in this work therefore have the same criteria of requirements as a commercial form.

References

Abbas T.F., Othman F.M., Ali H.B., 2017, Effect of infill Parameter on compression property in FDM Process, Int. Journal of Engineering Research and Application 7, 16-19

Chia H.N., Wu B.M., 2015, Recent advances in 3D printing of biomaterials, J. Biol. Engineering 9:4, 1-14

Compounding and the FDA, 2021, https://www.fda.gov/drugs/human-drug-compounding/compounding-and-fda-questions-ad-answers

Estrela M., Herdeiro M.T., Ferreira P.L., Roque F., 2020, The use of antidepressants, anxiolytics, sedatives and hypnotics in Europe: focusing on mental health care in Portugal and prescribing in older patients, Int. J. Env. Res. Public Health 17, 8612

Falconer J.R. and Steadman K.J., 2017, Extemporaneously compounded medicines, Australian prescriber 40, 5-8

Food and Drug Administration, 1997, Guidance for Industry Dissolution Testing of Immediate Release Solid Oral Dosage Forms

Gagliardi G.S., Shah A.P., Goldstein M., Denua-Rivera S., Doghramji K., Cohen S., 2009, Effect of Zolpidem on the sleep arousal response to nocturnal esophageal acid exposure, Clin. Gastroenterol. Hepatol. 7, 948-952

Glass J., Lanctôt K.L., Herrmann N., Sproule B.A., Busto U.E., 2005, Sedative hypnotics in older people with insomnia: meta-analysis of ricks and benefits, Br. Med. J. 331, 1169-1173

Goole J., Amighi K., 2016, 3D printing in pharmaceutics: A new tool for designing customized drug delivery systems, Int. J. Pharm. 499, 376-394

Gudeman J., Jozwiakowski, Chollet J., Randell M., 2013, Potential risks of pharmacy compounding, Drug 13, 1-8

Henry S., De Vadder L., Decorte M., Francia S., Van Steenkiste M., Saevels J., Vanhoorne V., Vervaet C., Developent of a 3D-printed dosing platform to aid in Zolpidem withdrawal therapy, Pharmaceutics, 13, 1684

ISO 5725-1, 1994, "Accuracy (trueness and precision) of measurement methods and results - Part 1: General principles and definitions.", p.1

Khachatryan S.G., 2021, Insomnia burden and future perspectives, Sleep Med. Clin. 16, 513-521

Korpela J., Kokkari A., Korhonen H., Malin M., Närhi T., Seppäla J., 2012, Biodegradable and bioactive porous scaffold structures prepared using fused deposition modeling, J. Biomed. Mat. Res. B: Applied Biomat. 101B, 610-619

Kripke D.F. Langer R.D., Kline L.E., 2012, Hypnotics' association with mortality or cancer: a matched cohort study, BMJ Open 2, e000850

Liang J-Z, 1995, Effect of the die angle on extrusion swell of rubber compound, J. Mat. Proc. Tech. 52, 207-212

Melchels F.P.W., Feijen J., Grijpma D.W., 2010, A review on stereolithography and its applications in biomedical engineering, Biomat. 31, 6121-6130

Minghetti P., Romanazzi L.C., Casiraghi A., Seklmin F., 2014, Are the quality and safety of magistral preparations guaranteed enough?, Pharmaceut. Reg. Affairs 3, 126

Mohanty S., Larsen L.B., Trifol J., Szabo P., Burri H.V.R., Canali C., Dufva M., Emnéus J., Wolff A., 2015, Fabrication of scalable and structured tissue engineering scaffolds using water dissolvable sacrificial 3D printed moulds, Mater. Sci. Eng. C 55, 569–578.

Mohiuddin A.K., 2019, Extemporaneous compounding: cautions, controversies and convenience, IJMHS 9, 252-264

Monti J.M., Spence D.W., Buttoo K., Pandi-Perumal S.R., 2017, Zolpidem's use in insomnia, Asian J. Psychiatry 25, 79-90

Murphy S.V., 2014, 3D Bioprinting of Tissues and Organs, Nat. Biotech. 32, 773-785

Orimo H., Ito H., Suzuki T., Araki A., Hosoi T., Sawabe M., 2006, Reviewing the definition of "elderly", Geriatr. Gerontol. Int. 6, 149-158

Perlis M.L., Morales K.H., Vargas I., Munch A.? Seewald M., Gooneratne N., Grandner M.A., Thase M.E., Kaptchuk T.J., Ader R., 2021, Durability of treatment to Zolpidem using partial reinforcement regimen: does this strategy require priming ?, Sleep Med. 87, 56-61

Pharmacy Board of Australia, Codes, Guidelines and Policies, 2015, Guidelines on compounding medicines

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

Poyares D., Pinto L.R., Tavares S., Barros-Vieira S., 2005, Hipnoindutores e insomnia, Rev. Bras. Psiquiatr. 27, 2-7

Norman J.L., Fixen D.R., Saseen J.J., Saba L.M., Linnebur S.A., 2017, Zolpidem prescribing practices before and after Food and Drug Administration required product labeling changes, SAGE Open Med. 5, 1-5

Sharf M.B., Mendels J., Thorpy M., Weiss B., 1994, Safety of long-term zolpidem treatment in patients with insomnia, Curr. Ther. Res. 55, 1100-1111

Siebern A.T., Suh S., Nowakowski S., 2012, Non-pharmacological treatment of insomnia, Neurotherapeutics 9, 717-727

Swaminathan Vidya and Dane O. Kildsig, Polydisperse Powder Mixtures: Effect of Particle Size and Shape on Mixture Stability, Drug Dev. Ind. Pharm. 28, 41-48

Touitou Y., 2007, Troubles du sommeil et hypnotiques : Impacts médicaux et socioéconomiques, Ann. Pharm. Franc. 65, 230-238

Unruh M.L., Hartunian M.G., Chapman M.M. et al., 2003, Sleep quality and clinical correlates in patients on maintenance dialysis, Clin. Nephrol. 59, 280-288

US Pharmacopoeia, Edition 42, Monography of Zolpidem Tartrate Tablets

Weaver M.F., 2015, Prescription sedative misuse and abuse, Yale J. Biol. Med. 88, 247-256

Winkelmayer W.C., Mehta J., Wang P.S., 2007, Benzodiazepine use and mortality of incident dialysis patients in the United States, Kidney Int. 72, 1388-1393

Xiang T., Cai Y., Hong Z., Pan J., 2021, Efficacy and safety of Zolpidem in the treatment of insomnia disorder for one month: a meta-analysis of randomized controlled trial, Sleep Med. 87, 250-256

Yang Y-W., Hsieh T-S., Yu C-H., Huang Y-S., Lee C-C., Tsai T-H., 2014, Zolpidem and the risk of Parkinson's disease: A nationwide population-based study, J. Psy. Res. 58, 84-88

Zhou Z., Buchanan F., Mitchell C., Dunne N., 2014, Printability of calcium phosphate: calcium sulfate powders for the application of tissue engineered bone scaffolds using 3D printing technique, Mat. Sci. Engin. C 38, 1-10