Department of Pharmaceutical Technology[1], Faculty of Pharmacy, Hacettepe University, Ankara; Department of Pharmaceutical Technology[2], Faculty of Pharmacy, İnönü University, Malatya, Turkey

Preparation and evaluation of furosemide containing orally disintegrating tablets by direct compression

T. Gulsun[1,#], N. Ozturk[1,#], M. S. Kaynak[2], I. Vural[1], S. Sahin[1,*]

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** Corresponding author: Prof. Selma Sahin, Department of Pharmaceutical Technology Faculty of Pharmacy, Hacettepe University, 06100, Sıhhiye, Ankara, Turkey sahin.selma@gmail.com*
#These authors contributed equally to this work.

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The purpose of this research was to develop and prepare orally disintegrating tablets (ODTs) containing furosemide by direct compression method. Furosemide, microcrystalline cellulose (MCC), low-substituted hydroxypropylcellulose LH-11 (L-HPC), aspartame, sodium stearyl fumarate were used for ODT formulation. MCC and L-HPC were used in ratios of 1:9 (ODT1) and 1:4 (ODT2). The results of the quality control parameters obtained for bulk powders (angle of repose, compressibility index, Hausner ratio, bulk density and volume, apparent density and volume, swelling of superdisintegrants and powder moisture) were taken as an indication of good compressibility of tablets. Both ODT1 and ODT2 disintegrated within 15 s and fulfilled the required disintegration time given by the European Pharmacopoeia (3 min). The average weight variation was less than 5% for both tablets. The friability of the tablets was less than 1%. Wetting time of both tablets was in the range of 12-21.7 s. Water absorption ratio was 1.41±0.03 for ODT1 and 1.96±0.10 for ODT2. Dissolution studies revealed that more than 85% of furosemide was dissolved in 15 min from both ODTs. Based on cell culture studies, permeability of furosemide was low (Papp=1x10^{-5} cm/s) but increased when prepared in the ODT form (ODT1: Papp=2x10^{-5} cm/s; ODT2: Papp=3.6x10^{-5} cm/s). Collectively, all these results showed that ODT formulations of furosemide were developed successfully. To improve patient compliance, ODT approach can be suggested for development and manufacturing of furosemide ODTs.

1. Introduction

The oral route is the most common route of drug administration for various pharmaceutical dosage forms (e.g. tablet, capsule, solution, suspension, emulsion, syrup) because of patient compliance, ease of administration, and safety (Valleri et al. 2004). The ODTs formulated to be dissolved on the tongue, are alternative dosage forms for patients with dysphagia. According to FDA (Food & Drug Administration), ODTs are "products designed to disintegrate or dissolve rapidly on contact with saliva, thus eliminating the need to chew the tablet, swallow an intact tablet or take the tablet with liquids" (FDA Guidance for Industry 2008). ODTs are also known as rapidly disintegrating, fast disintegrating, fast dispersing, rapid dissolving, fast dissolve/dissolving, rapid melting, fast melting, orodispersible tablets (Dobetti 2000; Habib et al. 2000; Sastry et al. 2000; Sunada and Bi 2002; Fu et al. 2004; Battu et al. 2007; Bandari et al. 2014). ODTs have many advantages including administration without the need of water, convenience and accuracy of dosing, simple production, pleasant taste, small packaging. Therefore, they are preferred to conventional tablets especially for geriatric, paralyzed, bedridden people, children, psychiatric patients (Hirani et al. 2009; Olmez and Vural 2009). Freeze drying (lyophilization), moulding, direct compression technologies, sublimation, spray drying, cotton candy, phase transition, granulation, three-dimensional printing, and mass extrusion methods are widely used for preparation of ODTs (Dobetti 2000; Fu et al. 2004; Goel et al. 2008; Prajapati and Ratnakar 2009; Bandari et al. 2014). In this study, ODTs were prepared by means of a direct compression method because it has many advantages such as low cost, just enough conventional equipment, less processing steps. In addition, high doses of drug and drugs which are sensitive to heat and moisture could be prepared by this method (Goel et al. 2008).

Furosemide is a potent loop diuretic used in the treatment of edematous states associated with congestive heart failure, cirrhosis of the liver, renal disease and chronic hypertension. The usual oral doses of furosemide are 20, 40 and 80 mg given as a single dose. According to the Biopharmaceutics Classification System (BCS) furosemide is a Class IV compound with low solubility and low permeability. Because of its weak acidic properties (pKa=3.8), furosemide is mostly absorbed in the stomach and upper intestine (Sweetman 2009), but also from the oral mucosa following sublingual administration (Haegeli et al. 2007). Bioavailability of furosemide is about 37 -70% (Klausner et al. 2003; Granero et al. 2010; Nielsen et al. 2015). The peak plasma concentration (C_{max}) is reached within 60 to 90 min (Granero et al. 2010). Furosemide is highly bound to plasma albumin (about 98%) (Basken et al. 2009). The plasma half-life ($t_{1/2}$) of furosemide is 1.3±0.8 h in healthy subjects (Klausner et al. 2003; Granero et al. 2010). The plasma concentration profile of furosemide after oral administration complies with the "flip–flop" type because the rate of absorption is slower than that of elimination (Garrison et al. 2015).

To our knowledge, no furosemide containing ODTs are commercially available. Therefore, the purpose of this study was to develop and prepare a furosemide ODT by means of a direct compression method. MCC (filler), L-HPC (superdisintegrant), aspartame (sweetening agent), sodium stearyl fumarate (lubricant) were used as inactive ingredients for preparation of ODTs. In the development of ODT formulations, MCC and L-HPC were used in ratios of 1:9 (ODT1) and 1:4 (ODT2) (Bi et al. 1996; Olmez et al. 2013). Quality control tests such as weight variation, diameter and thickness, friability, water absorption, resistance to crushing, disintegration time, and dissolution test were performed on ODTs. Permeabilities of bulk furosemide and in ODTs were determined across Caco-2 cell monolayers.

2. Investigations, results and discussion

2.1. Selection of excipients

The choice of excipients is very important for the development of a tablet formulation and also for the quality control of dosage forms. A very limited number of excipients is used for direct compression due to flowability, compressibility, and stability properties. MCC is widely used as filler in tablets but cause an increase in the dissolution rates and hardness of tablets compared to other fillers (Agrawal et al. 2016). However, this excipient has higher carrying capacity and it has unique compressibility properties. Due to its granular structure, it also has good flowability characteristics. Therefore, MCC was selected as filler in this study. Disintegration is also one of the most critical parameter for ODTs. Therefore, superdisintegrants are often used in the formulations. These substances are effective at low concentrations, show very high dispersing and a rapid swelling effect. Among them, L-HPC is widely used as a superdisintegrating agent at approximately 5–25% concentrations in the preparation of ODTs produced by direct compression methods (Rowe et al. 2009). L-HPC has some additional advantages such as excellent compatibility with active ingredients due to nonionic structure, disintegration into smaller particles for better dissolution due to high swelling capacity, and suitability for direct compression. Swelling capacity of L-HPC is reported to be higher than that of crospovidone, MCC and cornstarch (http://www.elementoorganika.ru/files/lhpc.pdf accessed 3 July 2016). Ishikawa et al. (2001) reported that MCC and L-HPC (1:9) containing ODTs were rapidly disintegrated (within 15 s). We therefore used L-HPC for the development of furosemide containing ODTs. ODTs disintegrate in the mouth in a short period of time, so that taste masking is very important especially for bitter tasting active ingredients. Aspartame is widely used as a taste masking agent in the commercially available ODT formulations such as MAXALT-MLT, Carinex®Redi Tabs®, Ultram®, Alavert®, REMERONsol TAB® and Children's Benadryl®. Therefore, aspartame was added to the furosemide ODT formulations as a taste masking agent at a ratio of 1% (Augsburger and Hoag 2008). Sodium stearyl fumarate which is an inert, hydrophilic direct compression tablet lubricant, was selected in our ODT formulations at a ratio of 1% because of its advantages including increased drug stability, faster disintegration, harder tablets compared to magnesium stearate, more suitable scale-up process, and less variability between the batches compared to magnesium stearate (http://www.jrspharma.com/pharmawAssets/docs/brochures/PRUV.pdf accessed 3 July 2016).

2.2. Physical properties of the powder blend

Flowability of powders is the most important feature for preparation of solid dosage forms. The powder must flow easily and uniformly for tablet weight uniformity and tablet production with consistent and reproducible properties. Various parameters such as bulk/tapped densities, angle of repose, are used for estimation of flowability of a powder blend. The bulk and tapped densities of a powder are described in Ph. Eur. 8th (European Pharmacopoeia 8th 2014) as "the ratio of the mass of an untapped powder sample and its volume including the contribution of the interparticulate void volume, and the tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample", respectively. Bulk and tapped volume/density values determined for the ODT powder blends are listed in Table 1. The bulk density values 0.35±0.01 for ODT1 and 0.37±0.01 g/mL for ODT2 were taken as an indication of good flowability. Angle of repose is also used for evaluation of flow behavior of a powder mixture. For this purpose, angle of repose of ODTs were calculated, and very similar values were obtained for ODT1 (39.3±1.4°) and ODT2 (41.9±2.4°) powder blends. Compressibility index values for ODT1 (17.5±5.4) and ODT2 (24.1±1.3) were slightly but not significantly different (Table 1). Both angle of repose and compressibility index values indicated that ODT1 has fair flowability, and ODT2 passable flowability characteristics according to Ph. Eur. 8th (European Pharmacopoeia 8th 2014). This difference in the flowability of ODT powder blends can be attributed to the L-HPC content of the formulations. It is reported that L-HPC has poor flowability properties (Diós et al. 2015). Therefore, with an increase in the L-HPC content of the powder blend, a decrease can be expected in the flowability. Our results confirm this expectation. In regard to angle of repose, in the literature, there is variety of reports available indicating that despite high angle of response values (i.e. 40-50°) formulation can be manufactured successfully (Pather et al. 1998; Ngwuluka et al. 2010; Ramasubramaniyan et al. 2013). In our study, a similar observation was obtained. Both ODT1 and ODT2 which have less than 45° angle of repose values were compressed successfully. The Hausner ratio could be used to evaluate to cohesiveness of powders. According to Ph. Eur. 8th, powders with Hausner ratios between 1.26 and 1.34, between 1.19 and 1.25 are considered to show passable flowability and fair flowability, respectively. The Hausner ratio values estimated that ODT2 powder blends show passable flowability and ODT1 powder blends show fair flowability (Table 1). These results were compatible with compressibility index and angle of repose values. The low moisture content is desirable for the flowability of powders for tableting. The moisture content determined in our study (3.3%, Table 1) is not expected to influence adversely the flowability of both powder blends during compression. Collectively, all these results showed that powders of ODT 1 and ODT 2 powder blends have suitable properties for tableting.

Table 1: Pre-compression parameters for bulk ODT powders (mean ± SD, n=3)

Parameter	ODT1	ODT2
Angle of repose (°)	39.3 ± 1.4	41.9 ± 2.4
Compressibility index	17.5 ± 5.4	24.1 ± 1.3
Bulk density (g/mL)	0.35 ± 0.01	0.37 ± 0.01
Bulk volume (mL)	142.5 ± 2.5	135.0 ± 2.0
Tapped density (g/mL)	0.43 ± 0.01	0.49 ± 0.01
Tapped volume (mL)	117.5 ± 7.1	102.5 ± 3.1
Moisture content (%)	3.1 ± 0.04	3.2 ± 0.3
Hausner ratio	1.21 ± 0.06	1.31 ± 0.05

2.3. Evaluation of tablets

Results of the quality control parameters performed on the developed ODT formulations are summarized in Table 2. As the diameter of tablets depends on the die and punches selected for the compression, low variabilities obtained in the tablet thickness and diameter values are consistent with the powder flow characteristics, and shows reproducibility of tablet compression.

Table 2: Quality control parameters of developed ODT formulations (mean ±SD)

Parameter	ODT1	ODT2
Weight variation (mg; n=20)	402.1 ± 3.4	401.2 ± 3.6
Friability (%; n=20)	0.2	0.4
Hardness (N; n=10)	75.1 ± 5.5	43.7 ± 6.2
Thickness (mm; n=10)	3.3 ± 0.0	3.4 ± 0.04
Diameter (mm; n=10)	11.8 ± 0.03	11.8 ± 0.04
Wetting time (s; n=3)	21.7 ± 4.5	12.0 ± 2.6
Water absorption ratio (n=3)	1.41 ± 0.03	1.96 ± 0.10
Disintegration time (s; n=6)	13	15

The resistance of tablets to abrasion and breakage during storage, transportation and handling before usage related to its hardness. The hardness of both ODT tablets (43.7±6.2 - 75.1±5.5 N) indicated good mechanical strength with an ability to withstand physical and mechanical stress conditions. These hardness values were suitable for ODTs and similar to literature values (2-6 kg/

cm^2) reported for ODTs (Kalia et al. 2009; Nagaich et al. 2010; Senthil et al. 2011).

According to Ph. Eur. 8th, orodispersible tablets should disintegrate within 3 min using conventional disintegration apparatus. Developed ODTs (ODT1 and ODT2) had very similar disintegration times (13 vs. 15 s) and fulfilled the Ph. Eur. 8th requirement (European Pharmacopoeia 8th 2014). These results also indicate that the amount of superdisintegrant (L-HPC) in the ODT formulation does not have a significant effect on the disintegration time.

According to Ph. Eur. 8th, no variation is desirable between individual tablets for uniformity, and up to 5% standard deviation is allowed. The weight variations (3.4 and 3.6%; Table 2) of the developed furosemide ODTs comply with the Ph. Eur. 8th requirement (European Pharmacopoeia 8th 2014).

Friability is an important quality control parameter for tablets that is necessary to guarantee tablet integrity during production, storage, and transport. According to Ph. Eur. 8th, friability values below 1% indicate a good tablet mechanical strength. Both ODTs fulfilled the Ph. Eur. 8th requirement for friability (Assaf et al. 2013; European Pharmacopoeia 8th 2014).

Wetting time of formulations was less than 22 s and water absorption ratios of formulations were 1.41±0.03 and 1.96±0.10 for ODT1 and ODT2 respectively. Wetting time and water absorption ratio values were slightly but not significantly higher for the ODT2 ($p>0.05$). These results obtained for ODT2 could be due to higher amount of L-HPC that is an effective disintegrant due to swelling and wicking actions in water (Mohanachandran et al. 2011).

In the furosemide tablet monograph, USP recommends that not less than 80% of the labeled amount is dissolved in 60 min (United States Pharmacopeia and National Formulary USP 36-NF 31 2013). According to the guidelines (FDA and EMA) (FDA Guidance for Industry 2000, EMA Guideline On The Investigation Of Bioequivalence 2010), if more than 85% of the labeled amount of drug substance in an immediate release drug product is dissolved within 30 min, dissolution profiles should be compared by a similarity factor (f_2). The similarity factor is a logarithmic reciprocal square-root transformation of the sum-of-squared error, and is a measurement of the similarity in the percent (%) dissolution between the two curves. Two dissolution profiles are considered similar if the f_2 value is ≥50. However, when 85% or more of the labeled amount of drug products dissolves in ≤15 min, dissolution profiles are considered similar without any mathematical calculation. Dissolution profiles of developed ODTs indicated that both ODT formulations released more than 85 % of the labeled furosemide amount within 15 min (Fig. 1). Therefore, dissolution profiles of ODT1 and ODT2 were considered similar without any mathematical calculation.

ODTs are designed to disintegrate rapidly in the oral cavity. In general, the majority of drug (furosemide) absorption takes place in the gastrointestinal tract after swallowing of ODTs. Furosemide was reported to be absorbed from oral cavity following sublingual administration (Haegeli et al. 2007). Compared to sublingual and buccal tablets, the residence time of ODTs in the mouth is very short, and hence furosemide absorption from the oral mucosa can be expected to be very low. Therefore, Caco-2 cells were used to study the effect of excipients on furosemide penetration in our study.

According to BCS, furosemide is a Class 4 compound with low solubility and low permeability. In the literature, furosemide permeability across Caco-2 cells was reported to be in between $0.086 - 0.466 \times 10^{-6}$ cm/s (Yamashita et al. 2000; Rege et al. 2001; Kerns et al. 2004). In the present study, all permeability values determined for bulk furosemide, and also both ODTs (Fig. 2) seemed to be higher than those of literature values. Although there was no statistically significant difference between the apparent permeability values of both ODTs and furosemide solution ($p>0.05$), furosemide in ODT2 formulation showed the highest permeability that those of ODT1 and bulk furosemide. This observation could be attributed to the higher amount of L-HPC in ODT2. In the literature, a similar observation was reported for 5-carboxyfluorescein. Although, permeability of this compound across rat jejunum and ileum was not influenced by different MCC concentrations (0.8 and 0.08 %), L-HPC (0.02 and 0.2 %) significantly increased permeability of 5-carboxyfluorescein across jejunum, but no influence on permeability across ileum (Takizawa et al. 2013).

ODTs disintegrate rapidly in the mouth, so the patient is exposed to the taste of the drug. Therefore, it is important to use taste masking agents when formulating bitter or unpleasant tasted drugs as ODTs to ensure patient compliance. Furosemide powder when tasted by the volunteers was found to have an unpleasant taste (Kawano et al. 2010b). Also furosemide has been subject of several taste masking studies (Kawano et al. 2010 a,b,c). In this regard, we used aspartame, a widely used taste masking agent in ODT formulations, in our formulations to mask unpleasant taste of furosemide.

When we compared the formulations, the main difference between the formulations is the superdisintegrant amount. Our aim was to prepare a rapidly disintegrating ODT with suitable hardness, dissolution and permeability properties. Effects of superdisintegrant amount on these parameters were investigated. ODT2 was expected to have a shorter disintegration time because of the higher superdisintegrant amount, however the results indicated that there was not a significant difference between two formulations in regard to disintegration time. This observation could be attributed to the conventional disintegration testing method we used. The large volume of water used for the test may have prevented us to observe the possible differences in the disintegration times. Wetting time is shorter and water absorption ratio higher for the ODT2 due to the higher amount L-HPC. This is an advantage over ODT1 because there is limited water in oral cavity for the disintegration to take place. MCC is more compressible than L-HPC (Bi et al. 1996). Therefore, ODT1 can be expected to have higher hardness due to the higher amount of MCC (Table 1). Our results seemed to confirm our expectations.

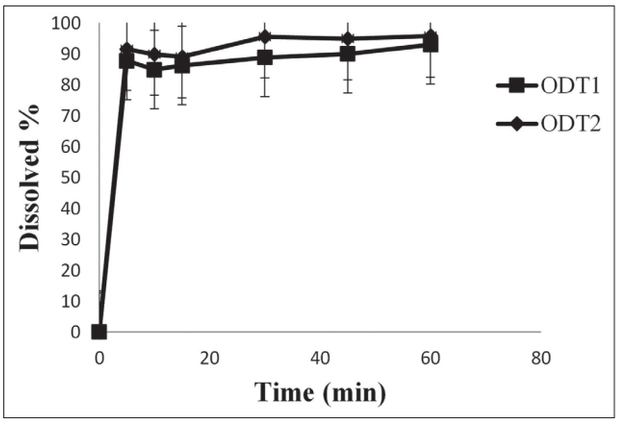

Fig. 1: Dissolution profiles of developed ODTs in pH 5.8 buffer (mean ± SD; n=6).

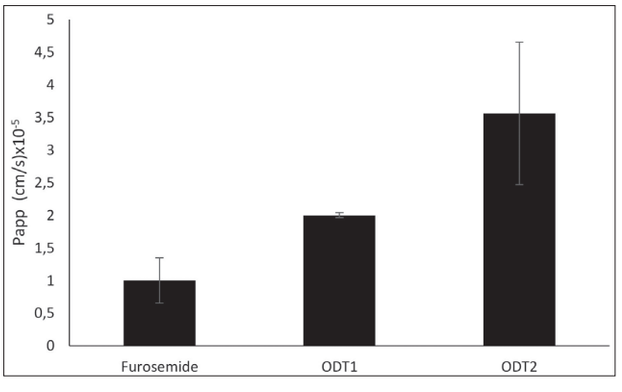

Fig. 2: Permeability values furosemide across Caco-2 cell monolayer (mean±SD; n=3).

In the literature, various methods including direct compression, combination of dry/wet granulation methods were used for preparation of furosemide containing ODTs (Koseki et al. 2008, 2009; Kawano et al. 2010 a, b, c; Perioli et al. 2012). Koseki et al. developed furosemide containing fast disintegrating tablets by a direct compression method using sucrose stearic acid ester (SSE) as a disintegration accelerating agent. In the presence of SSE, tablets were prepared by simple addition of SSE, using a lyophilized mixture of furosemide and SSE, or using a furosemide/SSE mixture obtained by evaporation of their ethanol solution. It was reported that only the tablets prepared with furosemide/SSE mixture obtained by ethanol evaporation had properties suitable for fast disintegrating tablets (hardness >30 N, and disintegration time < 20 s) (Koseki et al. 2008). Other fast disintegrating tablets were formulated using SSEs with a hydrophilic–lipophilic balance (HLB) of 16, 15 and 11. Before direct compression, a solvent evaporation method was used to obtain a furosemide/SSE/microcrystalline cellulose (MC) mixture, and then mixed with croscarmellose sodium and xylitol. It was demonstrated that an excellent fast-disintegrating tablet of furosemide could be obtained using homogeneous furosemide/SSE with HLB of 16/MC powder mixture (Koseki et al. 2009). Furosemide containing ODTs were prepared using taste-masked granules of furosemide containing maltitol, yogurt powder, or both (Kawano et al. 2010a,b,c). Two different approaches (dry or wet granulation) were used for the preparation of these granules. Taste-masked furosemide orally disintegrating tablets were then prepared by the direct compression method using Avicel PH-302 and mannitol as excipients. It was reported that the most rapidly disintegrating tablets (about 20 s) were obtained with yogurt granules prepared by dry granulation and furosemide at a mixing ratio of 1/1 (Kawano et al. 2010a). For the wet granulation method, it was concluded that furosemide ODTs with improved taste, rapid disintegration and greater hardness could be prepared with yogurt containing granules made by the wet granulation method using maltitol as a binding agent (Kawano et al. 2010b). In the case of granules containing both maltitol and yogurt powder prepared by the wet granulation method, it was reported that ODTs made from granules containing yogurt, maltitol and furosemide at a ratio of 0.5/0.5/1 respectively, exhibited the greatest hardness, the shortest disintegration time (within 30 s) and the shortest water absorption time (about 100 s) (Kawano et al. 2010c). Recently, furosemide was intercalated into the inorganic matrix hydrotalcite to obtain composites. Using this composite powder, furosemide tablets were prepared by direct compression with different superdisintegrants. Tablets, intended to be swallowed as a whole and to disintegrate rapidly in the stomach, have suitable hardness, disintegration time and they improved dissolution of drug in acidic medium (Perioli et al. 2012). When we compare our direct compression method with the other available methods, our method is simple, cost effective, and not time consuming. It can be prepared with easily found excipients, and does not require any additional step such as organic solvent evaporation, dry or wet granulation.

In conclusion, furosemide containing ODTs were developed successfully with an excellent disintegration time and dissolution profile. Both tablets fulfilled all pharmacopeial requirements, and more than 85% furosemide was dissolved within 15 min. When compared with conventional tablets, ODTs have a pleasant taste and easy processing. Therefore, to improve patient compliance, the ODT approach can be suggested for development and manufacturing of furosemide ODTs.

3. Experimental

3.1. Materials

Furosemide was kindly provided by Deva Holding A.Ş., Turkey. MCC (Avicel PH102), aspartame and sodium stearyl fumarate were all obtained from JRS Pharma, Germany, and L-HPC from Shin Etsu, Japan. The human colon carcinoma cell line Caco-2 cells were obtained from ATCC, USA. Dulbecco's Modifed Eagle's Medium (DMEM), Hank's balanced salt solution (HBSS), fetal bovine serum (FBS) were all purchased from Biochrom AG, Germany, and Penicillin-Streptomycin solution from Life Technologies, Inc., USA. Thincerts™ cell culture inserts (1.0 µm) were purchased from Grenier Bio-one, Germany. All other chemicals used in the study were of analytical grade.

3.2 Preparation of ODT formulations

Two different furosemide containing ODT formulations (ODT1 and ODT2) were developed using MCC as a filler, L-HPC as a superdisintegrant, aspartame as a sweetener, sodium stearyl fumarate as a lubricant. In these formulations, MCC and L-HPC were used in ratios of 1:9 (ODT1) and 1:4 (ODT2). Composition of furosemide containing ODT formulations were given in Table 3. Homogeneous physical mixture of MCC and L-HPC were prepared by mixing for 5 min in a roller mixer. Furosemide was added to this blend, and then mixed for a further 5 min. After thoroughly mixing with the sweetener, lubricant was added to the powder blend and mixed homogeneously for 5 min. The ODT tablets (400 mg) were compressed by direct compression method using Erweka AR 400, Germany.

Table 3: Composition of ODTs of furosemide

Ingredients (mg)	ODT1	ODT2
Furosemide	80	80
MCC	277.2	246.4
L-HPC	30.8	61.6
Aspartame	4	4
Sodium stearyl fumarate	8	8
Total weight	400	400

3.3 Characterization of ODT formulations

3.3.1. Physical properties of the ODT powder blends

The powder blend of each ODT formulation was characterized for bulk/tapped volume and density, compressibility index, angle of repose as suggested by the Ph. Eur. 8th (European Pharmacopoeia 8th 2014). Bulk/tapped volume and density were determined using a tap density powder tester (Aymes Company, Turkey). A weighed amount (50 g) of powder blend was filled into graduated cylinder of the apparatus. Unsettled (bulk) volume V_0 was read and noted. The cylinder was tapped 10, 500 and 1250 times and corresponding volumes V_{10}, V_{500} and V_{1250} were noted. If the difference between V_{500} and V_{1250} was greater than 2 mL, another 1250 taps were carried out and volume was noted as V_{2500}. The measurements were taken in triplicate, and each results were expressed as mean and the standard deviation. Bulk and tapped (settled) densities of the blend were calculated as follows.

$$Bulk\ density = m / V_0 \quad (1)$$

$$Tapped\ density = m / V_{1250\ (or\ 2500)} \quad (2)$$

where m is the weight of the sample (g) and V_0 is the bulk volume (mL), V_{1250} or V_{2500} (mL) is the tapped (settled) volume recorded after 1250 or 2500 tapping, respectively. Compressibility index of the powder mixtures was calculated using Eq. 3.

$$Compressibility\ Index = \frac{100\left(V_0 - V_f\right)}{V_0} \quad (3)$$

where V_f is the final tapped volume obtained for the ODT powder mixtures.
Hausner ratio of powder mixtures was calculated using Eq. 4.

$$\text{Hausner Ratio} = V_o / V_f \quad (4)$$

Flow characteristics of the powder blend were characterized by measuring the angle of repose which is related with the interparticulate frictions. For determination of the angle of repose, fixed funnel method was used. In this method, the powder blend is poured through a funnel to form a cone. The height of the resulting cone (h) is divided by half the width of the base of the cone (r), The inverse tangent of this ratio is defined as the angle of repose (Eq. 5).

$$\alpha = tan^{-1} h/r \quad (5)$$

The moisture content of powder mixture was also determined using a MB45 Moisture Analyzer (Ohaus Corporation, USA, Table 1) with a halogen dryer unit that ensures fast heating of the sample, and a balance. The sample (about 3 g) was heated at 105 °C with infrared heating unit until the weight of the sample was constant.

3.3.2. Evaluation of tablets

Quality control tests such as thickness, diameter and hardness, weight variation, friability, wetting time and water absorption ratio, disintegration and dissolution were performed on the ODTs.

3.3.2.1. Thickness, diameter and hardness determination

Thickness, diameter and hardness tests were performed on ODTs using Pharma Test PTB, Germany. Thickness, diameters (mm), and hardness (Newton; N) values were expressed as an average of 10 measurements.

3.3.2.2. Weight variation

Weight variation test was performed according to Ph. Eur. 8th (European Pharmacopoeia 8th 2014). Briefly, 20 tablets were chosen randomly and then weighed individually using an analytical balance. The mean weight was calculated, and individual tablet weight was compared to the mean weight.

3.3.2.3. Friability test

To determine the friability of ODTS, 20 tablets were weighed and then placed in a friability test apparatus (Pharma Test PTF, Germany). The tablets were rotated at 25 rpm for 4 min (100 revolutions). After 100 revolutions, the tablets were removed and weighed again. The weight was compared with the initial weight. The loss due to abrasion was taken as a measure of tablet friability and its value was expressed as the percentage. A maximum weight loss of not more than 1% is considered generally acceptable (European Pharmacopoeia 8th 2014).

3.3.2.4. Wetting time and water absorption ratio

For measurement of wetting time and water absorption ratio of ODTs, a piece of tissue paper folded twice was placed in a petri dish with a 10 cm diameter containing 6 mL of purified water. A previously weighed tablet was placed on the surface of the tissue paper, and the time to wet the tablet completely was noted as the wetting time. The fully wetted tablet was weighed and the water absorption ratio, R, was calculated as follows,

$$R = 100\ (W_a - W_b) / W_b \quad (6)$$

where W_b is weight of tablet before water absorption W_a is weight of tablet after water absorption (Bi et al. 1996).

3.3.2.5. Disintegration study

Disintegration test was performed according to the Ph. Eur. 8th with six tablets, and distilled water as the medium using Pharma test PTZ-S, apparatus (Germany). The time required for complete disintegration of each tablet was recorded individually. According to Ph. Eur. 8th, (European Pharmacopoeia 8th 2014) orodispersible tablets should disintegrate within 3 mins.

3.3.2.6. Dissolution study

In vitro dissolution tests for ODTs were undertaken according to the USP Pharmacopeia (United States Pharmacopeia and National Formulary USP 36-NF 31 2013). Dissolution test was performed on six ODTs in 900 mL of pH 5.8 phosphate buffer at 37±0.5 °C using Apparatus 2 (paddle method, 50 rpm; SOTAX). At predetermined time intervals (5, 10, 15, 20, 30, 45, 60 min), samples (2 mL) were withdrawn and replaced with fresh dissolution medium. The samples were filtered through 0.45 µm membrane filter and analyzed on UV spectrophotometer (Shimadzu 1800) at 274 nm to determine drug content.

3.3.2.7. Determination of furosemide permeability across Caco-2 cells

To evaluate the effect of excipients used on the permeability of furosemide, permeability of ODTs were determined across Caco-2 cell monolayer. Caco-2 cells (passage number 26) were cultured in DMEM supplemented with 10% FBS, 50 U/mL penicillin, 50 µg/mL streptomycin. Cells were seeded onto inserts (ThinCerts™; 1.0 µm) at a density of 60000 cells per well. Culture medium was changed every other day for 21 days until the cells achieve a consistent monolayer. Before the transport experiments, transepithelial electrical resistance (TEER) values were measured with a Millipore ERS voltameter, USA, and the cells with TEER values >300 $\Omega.cm^{-2}$ were used for permeability studies. The transport buffer was HBSS containing 25 mM D-glucose and 10 mM HEPES. A furosemide solution (50 µg/mL) was prepared in HBSS. For each ODT formulation, six tablets were powdered, and an equivalent amount of powder to produce 50 µg/mL furosemide concentrations was dispersed in HBSS. Furosemide solution and ODT formulations (0.5 mL) were added to apical (A) side, and blank transport buffer (1 mL) was added to the basolateral (B) side. After 2 hours incubation (at 37 °C, 60 rpm), transport buffer at the basolateral side was analyzed for drug content using UV spectrophotometer at 274 nm. The apparent permeability, P_{app} (cm/s) was calculated using Eq. (7).

$$P_{app} = \left(V_b C_b\right) / \left(C_0 A T\right) \quad (7)$$

where V_b is volume of transport buffer at basolateral side (cm^3), C_b is concentration of the drug at basolateral side , C_0 is initial concentration of the drug in the apical side (M), A is the surface area of insert (1.131 cm^2), and T is time period of experiment (s).

3.4. Statistical analysis

All tabulated results were expressed as mean±standard deviation (SD). Student's t-test was used in all statistical evaluation, and a p value less than 0.05 was considered significant.

Conflicts of interest: None declared.

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