International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 14, Issue 5, 2022

Original Article

FORMULATION AND CHARACTERIZATION OF ODT USING DIFFERENT CO-PROCESS CONTAINING DACLATSVIR: *IN VITRO* AND *IN VIVO* PHARMACOKINETICS STUDY ON HEALTHY VOLUNTEERS FOR HEPATITIS C TREATMENT

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Received: 31 Jan 2022, Revised and Accepted: 15 Jun 2022

ABSTRACT

Objective: This study aimed to prepare and evaluate oral disintegrating tablets (ODTs) of Daclatasvir dihydrochloride (DCV) using different co-processed excipients to enhance drug dissolution and improve oral bioavailability for the treatment of hepatitis C infection.

Methods: Ten Daclatasvir-ODTs formulae were prepared using co-processed excipients via direct compression. The prepared formulae were evaluated according to taste masking, weight variation, thickness, friability, hardness, drug content, and wetting time. *In vitro* disintegration time, *in vivo* disintegration time, and *in vitro* dissolution tests were also evaluated and taken as parameters for the selection of the best formula. The selected best formula was subjected to an *in vivo* study on volunteers and compared to a marketed product.

Results: All DCV-ODTs had acceptable physical properties in accordance with pharmacopeial standards. DCV-ODTs prepared with Pharmaburst® (F10) recorded the shortest wetting time (14±0.08s), fastest *in vitro* disintegration time (46±0.16s), shortest *in vivo* disintegration time (27±0.16s), and attained the fastest onset of dissolution (94.3±0.03 %) at 5 min to all other excipients and has been identified as the best formula. The *in vivo* pharmacokinetic study showed that the Pharmaburst-based formula has a significant Cmax increase of (2.17±0.28 µg/ml) compared to (1.42±0.59) for the marketed product and a significant decrease of Tmax to 60 min instead of 110 min for the marketed product.

Conclusion: The *in vivo* pharmacokinetic study in humans showed that the ODTs was found to be appropriate for delivery of Daclatasvir with a faster drug absorption rate when compared to the marketed products with applicable taste related to the nature of dosage form.

Keywords: Oral disintegrating tablets, Co-processed excipients, Daclatasvir dihydrochloride, Hepatitis C, Direct compression, *In vivo* pharmacokinetic study

DOI: https://dx.doi.org/10.22159/ijap.2022v14i5.44289. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Hepatitis C is an inflammation of the liver caused by the hepatitis C virus. The virus can cause both acute and chronic hepatitis, ranging in severity from a mild illness to a serious, lifelong illness, including liver cirrhosis and cancer. Globally, an estimated 58 million people have chronic hepatitis C virus infection, with about 1.5 million new infections occurring per year [1].

Hepatitis C is treated using direct-acting antiviral (DAA) tablets. DAA tablets are the safest and most effective medicines for treating hepatitis C. Direct-acting antivirals work by blocking the action of proteins which are essential for making new hepatitis C viruses.

Direct-acting antivirals available in the market, such as Daklinza® (daclatasvir), Sovaldi® (sofosbuvir), and Harvoni® (sofosbuvir/ledipasvir) are important medicines for treating chronic hepatitis C [2]. Daclatasvir (DCV) is a direct-acting antiviral agent that disrupts HCV replication by specifically inhibiting the critical functions of an NS5A protein in the replication complex. DCV is indicated for use with sofosbuvir, with or without ribavirin, for the treatment of chronic hepatitis C virus (HCV) genotype 1a/b or 3 infections. The dosing regimen of 60 mg Daclatasvir with 400 mg sofosbuvir once a day is recommended for both genotypes [3].

Oral disintegrating tablets (ODTs), also called orodispersible tablets, are uncoated tablets intended to be placed in the mouth, where they disperse rapidly before being swallowed [4, 5]. ODTs, by virtue of their unique characteristics, lead to better patient compliance, especially for pediatric and geriatric patients who often experience difficulty in swallowing. Dysphagia which is the medical term for swallowing difficulties, is estimated to afflict approximately six-million adults with 38% suffering from it for their entire lifetime [6].

Co-processing is a concept wherein two or more excipients interact at a sub-particle level to provide synergy in functionality and minimize drawbacks of individual excipients [7]. Excipients are the bulky components of pharmaceutical formulations. They had been appropriately assessed for safety and were included in a drug delivery system to support the processing of the drug delivery system within its production, enhance stability, bioavailability, patient acceptability or enhance any other attributes of the overall safety and effectiveness of the drug delivery system during storage or use [8]. Directly compressible co-processed excipient was the most largely explored method for the preparation of directly compressible adjuvants because it was cost-effective and could be prepared in-house based on the functionality needed [9]. In addition to that, they exert superior functionalities as compared to the physical mixture of the individual components. Examples include compressibility development and tablet strength, excellent anti-adherent ability and dissolution stability, enhanced resistance to lubricant sensitivity, enhanced flow properties, and reduced disintegration time [10].

Among co-processed excipient systems, Pharmaburst®, Ludiflash®, F-melt®, Prosolv® HD 90, Prosolv® SMCC 50, Prosolv® ODT G2, Prosolv® EASYtab SP, Prosolv® EASYtab Nutra, and Lactochem® Microfine. F-melt® is a spray-dried excipient used in orally disintegrating tablets that contain saccharides, disintegrating agents, and inorganic excipient. F-melt® displays excellent tableting properties and simplify rapid water penetration for a fast disintegration time [11]. Pharmaburst® is a fast-dissolving delivery system which incorporates addition of active drugs in a dry blend with Pharmaburst excipients before being compressed by tablet machine. It allows rapid dispersion and low adhesion to punches. Pharmaburst® is smooth and creamy and helps to mask the taste and grittiness of the API. Main advantage of Pharmaburst® is highly

compatible, has rapid disintegration and is cost-effective [12, 13]. Several co-processed cellulose excipients have allowed the decreasing of number of steps and the number of excipients needed in developing different formulations. Thus, simplifying the production processes, reducing costs and improving the dosage form properties. Therefore, the high functionality can be deemed to be terms of the improved processability in solid dosage forms such as (Prosolv® HD 90, Prosolv® SMCC 5O, Prosolv® ODT G2, Prosolv® EASYtab SP, and Prosolv® EASYtab Nutra) [14].

MATERIALS AND METHODS

Materials

Daclatasvir was a kind gift sample from Mesh Premiere (Cairo, Egypt). Pharmaburst® was provided by the SPI pharmaceutical company (Wilmington, DE, USA). Mannitol was obtained from Eruca sativa Freres reserves (Lestrem, France). Prosolv® ODT G2, Prosolv® HD 90, Prosolv® SMCC 50, Prosolv® EASYtab SP, Prosolv® EASYtab Nutra were obtained from JRS Pharmaceutical company GmbHand Co. KG (Rosenberg, Germany). F-melt® Type C was obtained from Fuji Industry Ltd. (Toyama-Pref, Japan). Lactochem® Microfine and Lactochem® Regular were obtained from (Borculo Domo Ingredients, Netherlands). A sweetening agent (sucralose/dextrose) was obtained from (Kamena, Egypt). Daklanork® 60 mg tablet (Mash Premiere Company, Egypt). Torsemide (Inad Pharma, Egypt). Instrument name: UPLC MS/MS "Waters" 3100 "USA".

Methods

Evaluation of flow properties of powder

Bulk and tapped densities

Powder was poured gently through a glass funnel into a graduated cylinder cut exactly to the 10 ml mark. The excess powder was removed using a spatula and the weight of the cylinder with pellets required for filling the cylinder volume was calculated. The cylinder was then tapped from a height of 10 cm until the time when there was no more decrease in the volume. Bulk density (ρb) and tapped density (ρt) were calculated [15].

$$\text{Tapped Density } (\rho t) = \frac{\text{Weight of sample}}{\text{Tapped volume}}$$

$$\text{Bulk Density } (\rho b) = \frac{\text{Weight of sample}}{\text{Bulk volume}}$$

Compressibility index and hausner ratio

Carr's index (CI) and Hausner ratio. Carr's index (Carr 1965) and Hausner ratio (Hausner 1967) for powders were calculated from bulk and tapped densities. Carr's compressibility index and the Hausner ratio provide a measure of the flow properties and compressibility of powders [16]. Carr's index (CI) and Hausner ratio for each formula were presented as a mean value±SD based on three measurements.

$$\text{Hausner's (ratio)} = \frac{\rho t}{\rho b}$$

$$\text{Carr}^{'}\text{s compressibility} = \frac{(\rho t - \rho b)}{\rho t} \times 100$$

Preparation of daclatasvir-ODTs

Direct compression method was used to prepare DCV-ODTs using ten types of co-processed excipients and a sweetening agent (3% Sucralose/Dextrose) to mask the better taste of Daclatasvir using a single punch tablet machine and a flat-faced 6 mm punch and die set. Finally, each ODTs was compressed to a final weight of 180 mg, containing 60 mg of Daclatasvir as shown in table 1.

Table 1: Composition of the prepared formulae

Ingredients (mg) formulae	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Daclatasvir (DCV)	60	60	60	60	60	60	60	60	60	60
Prosolv® EASYtab SP	118.4	–	–	–	–	–	–	–	–	–
Prosolv® EASYtab Nutra C	–	118.4	–	–	–	–	–	–	–	–
Prosolv® ODT G_2	–	–	118.4	–	–	–	–	–	–	–
F-melt® Type C	–	–	–	118.4	–	–	–	–	–	-
Prosolv® SMCC 50	–	–	–	–	118.4	–	–	–	–	–
Lactochem® Microfine	–	–	–	–	–	118.4	–	–	–	–
Mannitol	—	—	—	—	—	—	118.4	—	—	—
Prosolv® SMCC 90	–	–	–	–	–	–	–	118.4		
Lactochem® Regular	–	–	–	–	–	–	–	–	118.4	–
Pharmaburst®500	–	–	–	–	–	–	–	–	–	118.4
3% Sucralose/Dextrose	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Total (mg)	180	180	180	180	180	180	180	180	180	180

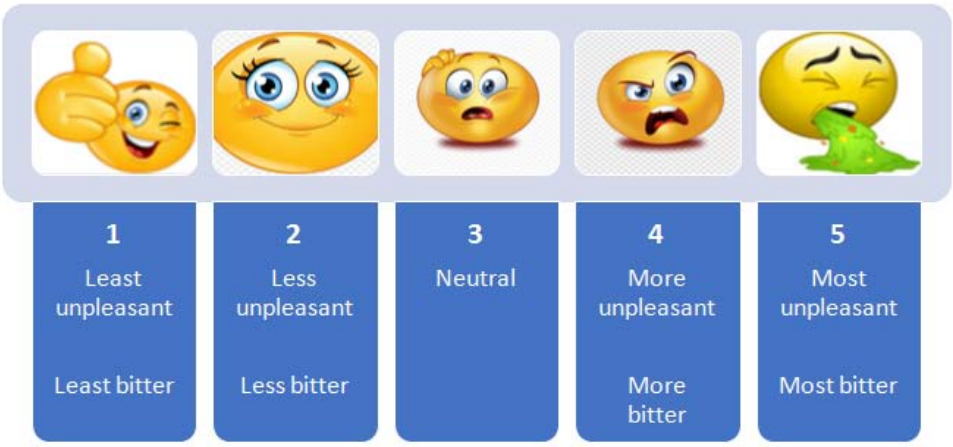

Fig. 1: Scoring system of daclatasvir-ODTs

Evaluation of prepared tablets

Taste evaluation

The taste of DCV ODTs and the plain drug was performed through six volunteers**.** All the volunteers were asked to rinse their mouths before tasting the tablet and were instructed not to swallow the tablet. The degree of taste masking of the ODTs formulae was evaluated by a selective taste panel using a scoring system. Six healthy male human volunteers were selected and feedback about the taste was obtained from all of them. After the taste evaluation test, all volunteers were supplied with drinking water [17].

Weight variation

Twenty tablets were selected randomly from each batch and their average weight was determined. Then each tablet was taken individually, and its weight was calculated. The individual tablets weight was compared with the average weight [18]. The findings were presented as a mean value±SD.

Thickness variation

Five tablets from each formulation were taken randomly and their thickness was measured using a micrometer (Starrett, Athol, MA), then the mean thickness and SD were calculated [19].

Friability test

A pre-weighed tablet was placed in the friabilator. Friabilator consists of a plastic chamber that revolves at 25 rpm, dropping those tablets at a distance of 6 inches with each revolution. The tablets then were rotated in the friabilator for at least 4 min. At the end of the test, tablets were dusted and reweighed; the loss in the weight of the tablet is the measure of friability and is expressed in percentage as the following equation:

$$\text{Friability percent} = \frac{\text{Loss in weight}}{\text{Initial weight}} \times 100$$ [20].

Hardness test

Hardness was measured for three ODTs from each formula using a tablet hardness tester to calculate the force required to break a tablet by compression in the radial direction [21].

Drug content

Ten tablets from each formula were assayed individually for drug content uniformity. The drug in ODTs was assayed by dissolving each tablet in distilled water. The solution was then filtered, properly diluted, and the absorbance was spectrophotometrically measured at λmax = 214 nm. Each individual tablet contents must be between 85-115% of the average content [22].

Wetting time

The wetting time (WT) was measured using circular tissue papers of 10 cm in diameter, which were placed in a petri dish of 10 cm diameter. Ten millilitres of water-soluble dye-like eosin solution was added to the petri dish. A tablet was carefully placed on the surface of the tissue paper. The time required for water to reach the upper surface of the tablet was noted as the wetting time. Any value of more than 3 min was considered a slow WT. The WT for each formulation was carried out in triplicate [23].

***In vitro* disintegration time**

Tablet was added to 10 ml distilled water at 37±0.5 °C. The time required for the complete dispersion of a tablet was measured. *In vitro* disintegration time (DT) for each formulation was determined in triplicate [24].

***In vivo* disintegration time**

The *in vivo* disintegration time was performed by human volunteers. Prior to the test, all volunteers were asked to rinse their mouths with distilled water. For the determination of the *in vivo* disintegration time of the prepared oral disintegrating tablets, each of six subjects was given a coded tablet. Tablets were placed on the tongue and immediately, the time was recorded using a stopwatch. They were allowed to move the tablet against the upper plate of the mouth with their tongue and to cause a gentle tumbling action on the tablet without biting on it or tumbling it from side to side. Immediately after the last noticeable granule had disintegrated, the time was recorded. The subjects were asked to spit out the content of the oral cavity after tablet disintegration and rinse their mouth with distilled water. The swallowing of saliva was prohibited during the test, and saliva was rinsed from the mouth after each measurement [25].

***In vitro* dissolution**

In vitro dissolution tests were performed with a dissolution tester, set with a paddle speed of 50 rpm, using 300 ml of distilled water at 37±0.5 °C as a dissolution medium. At specified time intervals (5,10,15,20,30,45,60 min) aliquots of 3 ml of dissolution media were withdrawn and replaced with an equal volume of the fresh medium drug content was assayed spectrophotometrically at 214 nm. Drug concentration was expressed as the cumulative percent drug dissolved. *In vitro* dissolution for each formulation was performed in triplicate [26].

Methodology of *in vivo* pharmacokinetic study of the best formula of DCV-ODT

The study was performed to compare the pharmacokinetics of a single dose of DCV-ODT formula (F10) and the reference, Daklanork® 60 mg tablet (Mash Premiere Company, Egypt) using a non-blinded, two treatments, two periods, randomized cross-over design. Under this design in period 1, half of the subjects were given the ODT formula, and the other half took the reference medicine. In period 2, the treatments were swapped. The study was approved by the Research Ethics Committee at the Faculty of Pharmacy, Cairo University (Serial no. of the protocol PI (2559) date 26/12/2019). The protocol complies with Helsinki and Tokyo declarations for humans. The research participants were aware of the study and the probable consequences or risks as adequate information about the research was given in a simple and easily understandable language. Measures to minimize the risks involved in the selection of the participants in addition to conducting the clinical research by competent and qualified persons. An informed consent form has been obtained from the participants which describe the clinical study, emphasizes the patient's role in decision-making, discusses the risks and the adverse effects of the medicines and the criteria of selecting the participants. All the data was kept confidential to prevent the disclosure of the identity of the involved participants.

Study design

Six healthy human male volunteers of ages between 18 to 55 y participated in the study. The health status of the volunteers was verified by a comprehensive medical history, physical checkup, and laboratory analysis for overall haematological and biochemical assessment, all these were carried out at the beginning. None of the volunteers had any history of drug or alcohol abuse, nor did they have any acute or chronic cardiac, gastrointestinal, vascular, renal, or hepatic disease. The subjects were directed to take no medicines for one week prior to and during the study; no coinciding medication was allowed during the study. Nicotine consumption was not allowed 10 h before and 24 h after drug intake, moreover, on each test day, caffeine and cola beverages were suspended from subjects 10 h before the taking of the medicines and till the blood sampling was finished. Each subject read, understood, and signed informed written consent. All subjects were notified about the risks and objectives of the study [27].

The health status of the volunteers was verified by complete medical history, physical examination, and laboratory analysis for complete haematological and biochemical examination. The subjects were instructed to take no medicines for one week prior to and during the study. The subjects were received in the facility at 8.00 am on the day of study after an overnight fast as instructed before the study. The subjects remained at the study site under controlled food and liquid intake till the end of the study day. Water was allowed freely 1 h post-administration. No food was allowed for four to five hours after dosing. The washout was one week. The subjects were under medical supervision during the study and were watched for any

adverse events such as headache, fatigue, nausea, vomiting, diarrhoea, insomnia, dizziness, or allergic reactions.

The ODT was administered orally without water, and each subject was asked to keep the ODT in the mouth for a few minutes until completely dissolved in the saliva, then, water was allowed after 30 min. The reference, Daklanork® 60 mg tablet was ingested with 240 ml of water [28].

Collection of blood samples

Blood samples (5.0 ml) were collected from a forearm vein into heparinized vacutainer tubes before administration of the dosage form at zero time (pre-dose), and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 24 h post-dosing.

The samples were assembled, and plasma was promptly separated from the blood cells by centrifugation at 3500 rpm for 10 min. The plasma obtained was stockpiled at −70 °C until analysis.

A washout period of 1 w was operated to remove the first intervention's effects [29]. A sensitive, selective, accurate and validated liquid chromatography-mass spectrometry (LC-MS/MS) method was used for the analysis of Daclatasvir-ODT and marketed Daclatasvir plasma samples. Torsemide was used as an internal standard. All assays were executed at ambient conditions [30].

Sample preparation

A volume of 50 µl of torsemide (from a stock solution with a concentration of 1200 ng/ml) was added to each sample (500 µl of the spiked human plasma samples) as an internal standard. The plasma sample and torsemide were extracted using Methanol (1 ml), vortexed for approximately 1 min, and then centrifuged for 15 min at 15,000 rpm at room temperature to permit protein precipitation. The supernatant was transferred to other vials filtered through a 0.22-µm membrane filter, then evaporated to dryness using a vacuum concentrator (Eppendorf Vacufuge plus, Germany). The dry residue was reconstituted in 0.5 ml of mobile phase and an aliquot of 10 µl of this solution was loaded into UPLC-MS/MS [31, 32].

Assessment of the pharmacokinetic parameters of daclatasvir

All pharmacokinetic parameters were estimated by non-compartmental analysis using Excel add-in PK-solver, and statistical analysis was done using the SPSS software. Pharmacokinetic parameters analysis includes: Cmax, Tmax, $T_{1/2}$, AUC $_{(0-24)}$, AUC $_{(0-\infty)}$, λ_z, and MRT were also calculated [33].

RESULTS AND DISCUSSION

Flow properties of powders

Pre-formulation study of blend powder of both drugs and their excipients flowability was done. The results of flowability of DCV in term of Carr's index (CI) was 45±0.04, which indicated very poor flow properties, while after homogenous blending of each drug powder with co-process excipients, the flowability improved, as shown in table 2. The best result for good flowability were for the blends including Pharmabrust® and F-melt® Type C in which CI was 13±0.021 [34].

Table 2: Flow properties parameters of powders; (Results are presented as mean±SD, n = 3)

	Tapped density±gm/ml	Bulk density±gm/ml	H-Ratio±(HR)	Carr`s index±(CI)
Plain drug: DCV	0.364±0.030	0.200±0.027	1.818±0.019	45±0.04
F1: Prosolv® EASYtab SP	0.506±0.023	0.420±0.019	1.205±0.021	17±0.012
F2: Prosolv® EASYtab Nutra C	0.593±0.031	0.433±0.035	1.370±0.017	27±0.026
F3: Prosolv® ODT G_2	0.767±0.011	0.637±0.015	1.205±0.021	17±0.023
F4: F-melt®Type C	0.644±0.013	0.560±0.011	1.149±0.015	13±0.021
F5: Prosolv® SMCC 50	0.466±0.028	0.340±0.025	1.370±0.029	27±0.023
F6: Lactochem® Microfine	0.616±0.019	0.450±0.014	1.370±0.021	27±0.018
F7: Mannitol	0.500±0.021	0.300±0.019	1.667±0.023	40±0.019
F8: Prosolv® SMCC90	0.658±0.021	0.480±0.021	1.370±0.020	27±0.015
F9: Lactochem® Regular	0.417±0.020	0.300±0.022	1.389±0.029	28±0.021
F10: Pharmabrust®500	0.569±0.031	0.495±0.029	1.149±0.034	13±0.021

Table 3: Scoring results of the bitter taste for DCV-ODTs

Formulae	Score	Meaning
Plain drug: DCV	5	Most bitter
F1: Prosolv® EASYtab SP	3	Neutral
F2: Prosolv® EASYtab Nutra C	2	Less bitter (less unpleasant)
F3: Prosolv® ODT G_2	3	Neutral
F4: F-melt®Type C	3	Neutral
F5: Prosolv® SMCC 50	3	Neutral
F6: Lactochem® Microfine	2	Less bitter (less unpleasant)
F7: Mannitol	3	Neutral
F8: Prosolv® SMCC 90	3	Neutral
F9: Lactochem® Regular	3	Neutral
F10: Pharmabrust® 500	2	Less bitter (less unpleasant)

Improve of the bitter taste of daclatasvir

The drug alone showed the most bitter taste. All prepared ODTs had a neutral to less bitter taste compared with the plain drug as shown in table 3. The ODTs containing Prosolv® EASYtab Nutra C, Lactochem® Microfine, and Pharmabrust had the most pleasant taste according to the scoring system.

Characterization of the prepared formulae

The weight of different ODTs ranged from (175±0.08 mg, F8) to (182±0.08 mg, F3). All formulations were within the British Pharmacopeia specification for drug content and for weight variation, and none of the tablets deviated from the average weight by more than 7.5%. The prepared tablet showed the same thickness (3.33±0.00).

Friability testing is used to determine the physical strength of compressed and uncoated tablets upon exposure to mechanical shock and erosion. It shows how much mechanical stress tablets can endure during their manufacturing, packaging, distribution and handling by the customer. The strength of a tablet plays a very important role in its marketing and dissolution. The generally agreed upper limit for friability is 1% [20]. All ODTs did not break or show any capping, cracking, or chipping during the test. The

friability of them was found within the desirable range, except for Lactochem® Microfine (F6), which was 1.8%.

The hardness of ODTs is usually desirable between 2 and 8kg [20]. The hardness of tablets depends on the compression force and the amount and type of binding agent present. The compression force used was the same for all formulations; therefore, the change in hardness values of different tablets observed in table 4 could be referred to the amount and type of binding agents in the co-process excipients. The hardness of the ODTs was found to be about 3.4±0.21 to 9.4±0.74 kg, which can provide adequate strength and porosity to ensure short wetting and disintegration time of the tablets.

Table 4: Physical evaluation of the ODT formulations (Results are presented as mean ± SD, n is stated in each test column of results)

Formulae	Weight (mg); n = 20	Thickness (mm); n = 5	Friability (%)	Hardness (Kg); n = 3	Drug content (%); n = 10	*In vitro* DT (sec); n = 3	*In vivo* DT (sec); n = 6	WT (sec); n = 3
F1	181±0.08	3.33±0.00	0.14	5.8±0.63	85.3158±0.21	57±0.15	90±0.14	47±0.06
F2	176±0.07	3.33±0.00	0.9	9.4±0.74	104.47±0.01	42±0.26	28±0.43	195±0.18
F3	182±0.08	3.33±0.00	0.27	3.4±0.54	85.00±0.03	205±0.17	207±0.22	100±0.09
F4	179±0.05	3.33±0.00	0.08	4.7±0.23	100.53±0.021	191±0.18	36±0.20	116±0.26
F5	180±0.06	3.33±0.00	0.16	7.7±0.36	91.57±0.05	385±0.26	190±0.10	173±0.32
F6	177±0.07	3.33±0.00	1.8	6.7±0.83	105.78±0.02	160±0.09	175±0.30	76±0.33
F7	179±0.06	3.33±0.00	0.93	3.6±0.21	84.47±0.05	267±0.14	175±0.11	177±0.25
F8	175±0.08	3.33±0.00	0.12	5.7±0.44	88.94±0.07	551±0.10	213±0.21	100±0.13
F9	179±0.09	3.33±0.00	0.92	4.9±0.54	90.78±0.01	173±0.28	136±0.14	20±0.11
F10	178±0.07	3.33±0.00	0.87	3.4±0.21	85.94±0.02	46±0.16	27±0.16	14±0.08

Note: Data are presented as mean value±SD. Abbreviations: WT, wetting time; DT, disintegration time; n, number of experiments.

Wetting time

Wetting time (WT) is a key parameter that gives an indication of the disintegration properties of the tablets. Shorter WT usually implies a faster disintegration. Time limit of 180s was set, and a higher value was deemed inconvenient for an ODTs [23]. The results of WT shown in table 4 revealed that 90% of the prepared DCV-ODTs had acceptable WT. Pharmaburst® (F10) recorded the shortest WT (14±0.08s) while Prosolv® EASY tab Nutra C-based formula (F2) recorded the longest WT (195±0.18s) that exceeded the defined limit. Pharmaburst® has a mixture of sorbitol and mannitol which promotes hydration compared to Mannitol alone (F7) which has a WT (177±0.25). The favorable hydration capacity of sorbitol occurred due to the presence of equatorial OH on the C-2 atom, which has the preference to have two hydrogen-bonded contacts, resulting in a high wetting capacity and hence lowering the WT compared to mannitol, which contains an axial OH on the C-2 atom that tends to have only one hydrogen bond. Our results are in harmony with the work reported by Teaima MH *et al.*, on Pitavastatin Calcium and Lornoxicam oral disintegrating tablets [35].

***In vitro* and *in vivo* disintegration time**

Table 4 exhibits the *in vitro* and *in vivo* DT results of all ODTs. According to the European Pharmacopeia, the limit for the DT of ODTs is 3 min [36]. As a result, most of the ODTs formulas had acceptable DT values. The results revealed that Prosolv® EASYtab Nutra C (F2) and Pharmaburst® (F10) had significantly the fastest DT, while Prosolv® SMCC 90 (F8) had the longest DT when compared with other formulae. This may be due to the presence of Microcrystalline cellulose (MCC) in Prosolv® SMCC 90, which lowers the water uptake into the tablet and lead to the disintegration delay.

Although Pharmaburst®, Prosolv® ODT G2, and F-melt® consist of crospovidone as a superdisintegrant, they had different DT values. This could be attributed to the presence of a mixture of sorbitol and mannitol in Pharmaburst® as previously mentioned [35]. The same justification was given for Prosolv® ODT G2 (F3), as it also contains mannitol alone. Moreover, it has a complex matrix (crospovidone, microcrystalline cellulose (MCC), mannitol, and fructose), which might be the reason for its disintegration delay. In addition, to the presence of MCC like Prosolv® SMCC 90. When it comes to F-melt® (F4), its disintegration delay could be attributed to the fact that it had less surface area than Pharmaburst® [36]. Our result complies with those of Moqbel *et al.*, who reported that ODTs prepared using Prosolv® ODT G2 and F-melt® showed delayed DT in contrast to Pharmaburst® in preparation of chlorzoxazone ODTs [36]. The same justification was applied for Lactochem® Microfine (F6) as it contains highly processed lactose. The results of *in vivo* and *in vitro* disintegration time showed a good correlation between both of them and the wetting time. Pharmabrust® as a co-process showed the least time of disintegration either *in vivo* or *in vitro* correlated with the least value of wetting time

***In vitro* dissolution study**

Pharmaburst (F10) attained the highest 94.3±0.03 % dissolved drug at 5 min, and 96.2±0.09 % dissolved drug at 10 min, followed by Lactochem® Regular (F9) then Mannitol (F7). The results were in accordance with those obtained from WT and DT as shown in fig. 2.

Fig. 2 displays the *in vitro* dissolution profile of DCV from the different prepared ODTs. The amount of DCV dissolved after 5 min (Q5 min) and 10 min (Q10 min) were taken as a parameter for comparison between the different ODTs. The results showed that Pharmaburst® (F10) attained the highest 94.3±0.03 % dissolved drug at 5 min, and 96.2±0.09 % dissolved drug at 10 min, followed by Lactochem® Regular (F9) then Mannitol (F7). The results were in accordance with those obtained from WT and DT. The highest % DCV dissolved recorded by Pharmaburst® might be due to the higher capacity of crospovidone as a superdisintegrant, as it had high capillary activity and marked hydration with little propensity for gel-formation [36]. Furthermore, crospovidone particles are granular and highly porous, allowing water to wick into the tablet, resulting in rapid disintegration, and thus enhancing the drug dissolution.

Statistical design for optimization of ODT

Optimization of ODT was done by Design expert software (Version 13, Stat. Ease Inc. and Minneapolis, MN, USA). General factorial design with one factor (X1), 10 levels and 20 runs were selected for the optimization study. The ODTs were prepared with different co-proceeded excipients and responses hardness (y1), friability (y2), *in vitro* dissolution percent after 5 min (y3), *in vitro* disintegration time (y4), wetting time (y5) and *in vivo* disintegration time (y6).

Among the various formulations, optimum formulations were selected based on the desirability factor. Criteria for the selection were primarily based upon the highest possible values of *in vitro* dissolution percent, the lowest possible values of hardness, friability, *in vitro* disintegration time, wetting time and *in vivo* disintegration time. Based upon previous parameters Pharmaburst-based formula (F10) was selected as the optimal formula.

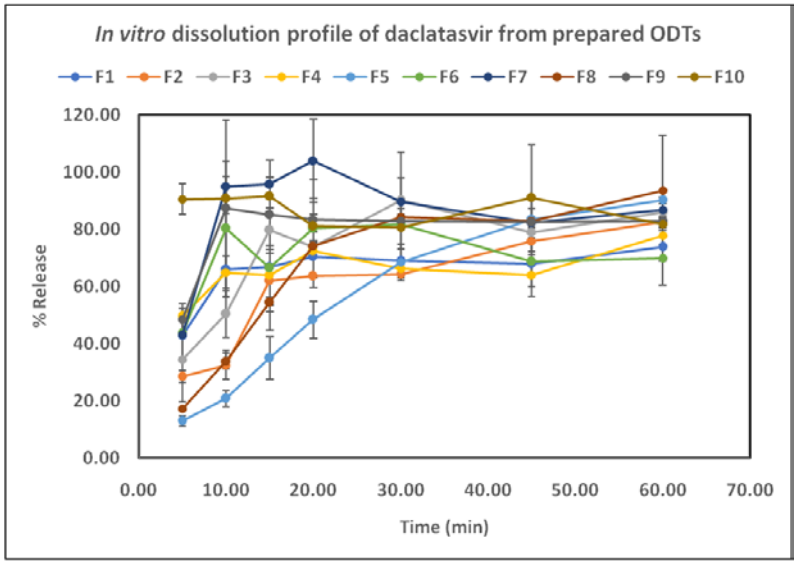

Fig. 2: *In vitro* dissolution profile of daclatasvir from prepared ODTs, results are the mean of 3 experiments, error bars are the SD

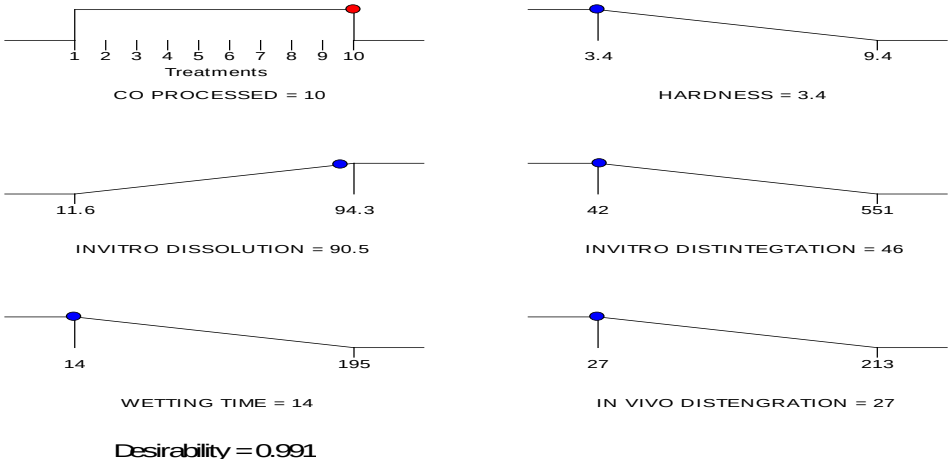

Fig. 3: Graphical illustrations of optimal formula

Assessment of *in vivo* pharmacokinetic parameters

The study was conducted on the six volunteers who were included in the pharmacokinetic analysis. The volunteers sustained excellently with the two treatments and did not complain of any adverse effects during the study. No signs of headache, gastrointestinal disturbances, or allergic reactions were witnessed from any of the volunteers during the study. A one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) as a post hoc test was applied using SPSS program version 17 software. The differences were considered significant if P<0.05. Table 5 and fig. 4 showed the Pharmacokinetic parameters of the Daclatasvir-ODT formula (Test) and the commercial brand Daklanork® (Reference) following oral administration. It was noticed that Cmax of the prepared formula F10 (Pharmaburst®) was about 2 folds of the brand commercial product and Tmax of the brand was 2-fold of the Tmax of F10.

Table 5: Pharmacokinetic parameters of the daclatasvir-ODT (Test) and commercial brand (Reference) following oral administration, results are presented as mean±SD, n = 6

	(Reference) Daklanork®-Brand commercial product	Test (Daclatasvir-ODT)
Cmax (ng/ml)	1420.643±591.747	2172.363±277.606
AUC0-t (h. ng/ml)	11756.993±4423.456	9573.872±3209.271
AUC0-∞ (h. ng/ml)	17152.365±8000.635	13456.08±5121.497
Tmax (h)	1.833±0.258	1±0
AUMC 0-t (h2. ng/ml)	103533.398±43124.65	79596.132±33807.261
AUMC 0-∞ (h2. ng/ml)	355663.807±248253.467	249863.744±131528.961
T 1/2 (h)	13.481±4.351	13.066±3.67
MRT (h)	8.713±0.726	8.092±0.997
λ Z(1/h)	0.056±0.019	0.057±0.017

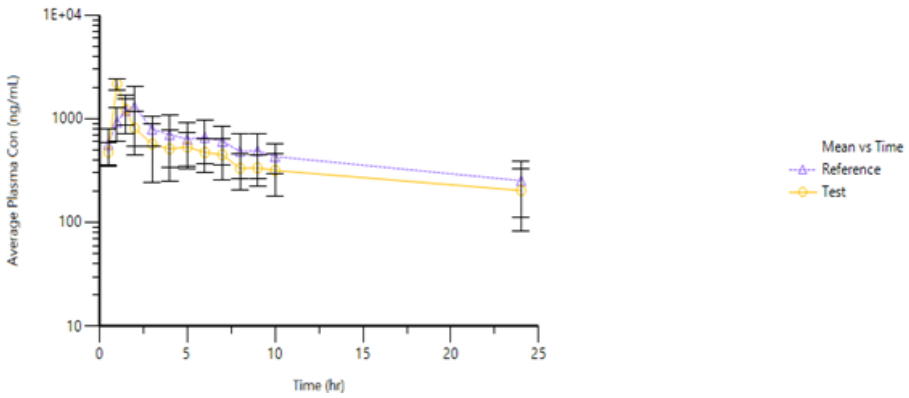

Fig. 4: Mean plasma concentration profiles of the daclatasvir-ODT (F10) and commercial brand (Reference) following oral administration, results are the mean of 6 subjects with error bars are the SD

CONCLUSION

Pharmaburst was suitable to form fast-disintegrating tablets by direct compression method, also considered a co-process excipient that showed the optimum results of ODTs evaluation test comparatively with other excipients, it can provide the pharmaceutical producer with multifunctional property with cost-saving in drug technology. Daclatasvir-ODT using Pharmaburst as a co-process is a promising dosage form in hepatitis C treatment in terms of enhancement of drug dissolution and chemical bioavailability compound by the marketed product since the formulated Tmax was decreased and Cmax was increased.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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