Studies on Development and Evaluation of Carbopol Based Pellets of Selected Drugs Using Extrusion-Spheronization Technology

THESIS

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by

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under the supervision of
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CERTIFICATE

This is to certify that the thesis entitled "**Studies on Development and Evaluation of Carbopol Based Pellets of Selected Drugs Using Extrusion-Spheronization Technology**" submitted by **Satish Sangappa Reddi**, ID. No. **2011PHXF0410P** for award of PhD degree of the institute, embodies original work done by him under my supervision.

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Satish Sangappa Reddi

Abbreviations and Symbols

#	Mesh
%	Percentage
%, w/w	Percentage weight by weight
%, w/v	Percentage weight by volume
% CDR	Percentage cumulative drug release
% RSD	Percentage relative standard deviation
% RTD	Percentage remaining to be degraded
λ_{max}	Wavelength of maximum absorbance
<	Less than
>	More than
≤	Less than equal to
≥	More than Equal to
=	Equal to
≈	Approximately equal to
σ	Standard deviation of y intercept of regression equation
$^{\circ}C$	Degree centigrade
ΔH	Enthalpy
ρb	Bulk density
ρt	Tapped density
3D	Three dimensional
$^{\circ}C\ min^{-1}$	Degree centigrade per minute
µm	Micrometer
µl	Micro liter
$\mu g\ ml^{-1}$	Micro gram per milliliter
$\mu g\ l^{-1}$	Microgram per liter
$\mu g\ h\ l^{-1}$	Micro gram hour per liter
ACN	Acetonitrile
ANOVA	Analysis of variance
API	Active pharmaceutical ingredient
AT	Accelerated temperature
ATR	Attenuated total reflectance
AUC	Area under curve
AUMC	Area under the first moment curve
$AUC_{(0-\infty)}$	Area under plasma concentration-time curve
BBD	Box-Behnken design
BCS	Biopharmaceutical classifications system

d_g	Geometric mean diameter on weight basis
cm	Centimeter
cm^2	Centimeter square
C_{max}	Maximum concentration
Conc.	Concentration
cps	Centipoises
CR	Controlled release
CRT	Controlled room temperature (25 ± 2 ºC/60 ± 5 % RH)
DC	Drug content
DCP	Di-calcium phosphate
DOE	Design of experimentation
DSC	Differential scanning calorimetry
EMA	European medicine agency (EMA)
FFD	Fractional factorial design
F_r	Relative bioavailability
FT	Refrigerated temperature (5 ± 2 ºC)
FTIR	Fourier transform infra-red
g	Gram
g l^{-1}	Gram per liter
GC	Gas chromatography
GERD	Gastro-oesophageal reflux disease
GI	Gastrointestinal
GIT	Gastrointestinal tract
hr	Hour
HCl	Hydrochloric acid
HEC	Hydroxyethyl cellulose
HETP	Height equivalent to theoretical plates
HPLC	High performance liquid chromatography
HPLC-MS/MS	High performance liquid chromatography-tandem mass spectrometry
HPTLC	High performance thin layer chromatography
HPMC	Hydroxy propyl methyl cellulose
HPC	Hydroxypropyl cellulose
HQC	Higher quality control sample
ICH	International conference on harmonization
I.M.	Intramuscular
IP	Indian Pharmacopoeia

IR	Immediate release
IS	Internal standard
i.v.	Intravenous
J g^{-1}	Joule per gram
K	Release rate constant for 'Korsmeyer-Peppas' empirical equation
K_0	Zero order release rate constant
K_1	First order release rate constant
K_{deg}	Degradation rate constant
Kg	Kilogram
kg cm^{-2}	Kilogram per square centimeter
K_H	Release rate constant representative of square root kinetics
l	Liter
l day^{-1}	Liter per day
l kg^{-1}	Liter per kilogram
LCMS	Liquid chromatography coupled with mass spectrophotometer
l $h^{-1}kg^{-1}$	Liter per hour per kilogram
l kg^{-1}	Liter per kilogram
LLOQ	Lower limit of quantification
LOD	Limit of detection
log % RTD	Log percentage remaining to be degraded
log P	Log of oil water partition coefficient
LOQ	Limit of quantification
LQC	Lower quality control
M	Molar
MCC	Microcrystalline cellulose or cellulose microcrystalline
MDT	Mean dissolution time
mg	Milligram
mg day^{-1}	Milligram per day
mg ml^{-1}	Milligram per milliliter
min	Minute
ml	Milliliter
ml min^{-1}	Milliliter per minute
mM	Millimolar
mm	Millimeter
mm sec^{-1}	Millimeter per second
MQC	Medium quality control
MRT	Mean residence time

MSC	Model selection criterion
MW	Molecular weight
M_t/M_∞	Fraction of drug released at time t
MΩ.cm	milliohm centimeter
N	Newton
n	Diffusional exponent indicative of release mechanism in Korsmeyer-Peppas model
NDDS	Novel drug delivery systems
ng ml^{-1}	Nanogram per milliliter
NIR	Near infra-red
nm	Nanometer
PBS	Phosphate buffer saline
PD	Parkinson's disease
pH	Negative log to the base 10 of hydrogen ion concentration
pKa	Acid dissociation constant
PRESS	Predicted residual sum of squares
p-value	Significance level in statistical tests (probability of a type I error)
PVP	Povidone or Polyvinylpyrrolidone
$P_{o/w}$	Equilibrium partition coefficient
QC	Quality control
R^2	Regression coefficient
R_f	Retention factor
RH	Relative humidity
RMG	Rapid mixer granulator
RP-HPLC	Reverse phase-high performance liquid chromatography
rpm	Revolutions per minute
Rs	Resolution
RSD	Relative standard deviation
R_t	Retention time
S	Slope of the least square regression line
SD	Standard deviation
Sec	Seconds
SEM	Scanning electron microscopy
SPE	Solid phase extraction
ssNMR	Solid state nuclear magnetic resonance spectroscopy
T	Temperature
Tf	Tailing factor

$t_{1/2}$	Half-life
$t_{50\%}$	Time to reach 50% of initial concentration
$t_{90\%}$	Time to reach 90% of initial concentration
T50%	Time taken for 50% of drug release from formulations
T80%	Time taken for 80% of drug release from formulations
t_{Cal}	Calculated t-values
t_{Crit}	Critical t-value
TDW	Triple distilled water
T_m	Melting temperature
TLC	Thin layer chromatography
T_{max}	Time taken to reach maximum concentration
USA	United States of America
USFDA	United States Food and Drug Administration
USP	United States Pharmacopoeia
UV	Ultra Violet
Vd	Apparent volume of distribution
Vis	Visible
Vss	Apparent volume of distribution at steady-state
v/v	Volume by volume
v/v/v	Volume by volume by volume
w/w	Weight by weight
XRD	X-ray diffraction method

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Abstract

Carbopols are high molecular weight, cross-linked, acrylic acid based polymers which have outstanding release retarding properties. Use of these polymers may allow us to use extrusion-spheronization technology to prepare controlled release matrix pellets. Compared to currently used methods for the preparation of controlled release pellets, i.e. powder layering and fluidized bed technology, extrusion-spheronization is a single step, rapid, operator independent and precise method. Also, pellets manufactured with extrusion-spheronization technology are dense and hard, making them more suitable for controlled release formulations. Furthermore, use of Carbopols may impart additional functions such as taste masking, binding, bioadhesion, etc. and may also increase bioavailability. Therefore, Carbopol polymers along with extrusion-spheronization technology can be utilized for designing of improved drug delivery system with lower cost. Owing to these manufacturing and therapeutic advantages, the primary objective of the present research work was to develop and evaluate pellet formulations with extrusion-spheronization technology using Carbopol polymers. Solubility of the drug has a substantial impact on designing extended release formulation as well as the extrusion-spheronization process. Thus, to study the wide range applicability of Carbopols, a low soluble drug (domperidone) and a high soluble drug (venlafaxine hydrochloride) were selected as model drugs for the present research work.

In order to assess the quality of formulation, suitable analytical methods are essential at each stage of formulation development. As per the need of present research work, new UV spectroscopic methods and stability indicating liquid chromatographic methods were developed and validated according to regulatory guidelines. These analytical methods were used for the estimation of drugs during various preformulation studies and formulation studies. In addition, bioanalytical method was developed for the estimation of domperidone in rat plasma using solid phase extraction technique. Further, developed bioanalytical method was successfully applied for the estimation of domperidone during in-vivo pharmacokinetic studies of the pure drug and pellet formulations in rats. Prior to the formulation development, various preformulation studies including solubility, stability and drug-excipient compatibility studies were performed to establish necessary physicochemical data of the domperidone and venlafaxine hydrochloride.

Controlled release matrix pellets of domperidone were designed and developed using Carbopol polymers with extrusion-spheronization technology without addition of any electrolytes. More than 85% yield was achieved with suitable shape and size distribution. The drug release was extended up to 24 hr. Furthermore, in vitro release profile of these newly developed matrix pellets was successfully matched with domperidone pellets prepared using fluidized bed technology. High drug (up to 60% w/w) loaded pellets were also prepared for venlafaxine hydrochloride using Carbopol polymers with more than 90% yield. Various formulation variables and process parameters involved in extrusion-spheronization to develop Carbopol based pellets were screened using fractional factorial designs. Also, optimization studies were carried out using Box-Behnken design studies. Optimized pellets of both the drugs were found to possess acceptable physical characteristics indicating the suitability of the manufacturing technique adopted. The assay values and in vitro release studies of all the designed formulations were found to be highly satisfactory. The designed pellet formulations for both domperidone and venlafaxine hydrochloride were found to be stable during stability studies designed as per ICH and WHO guidelines.

Pharmacokinetic studies in rats were carried out for domperidone administered orally in the form of suspension, immediate release pellets (pellets without Carbopol 971) and controlled release pellets (pellets with Carbopol 971). In vivo performance of controlled release pellet formulation was compared against an immediate release pellet formulation. The delayed T_{max}, reduced C_{max} and prolonged $t_{1/2}$ confirmed controlled release character of the designed Carbopol based pellets formulation of domperidone.

The results obtained by the current research indicated that Carbopol based pellets can be successfully prepared using extrusion-spheronization without addition of electrolytes. This Carbopol based matrix pellet preparation method is simple, cost-effective, operator independent and requires very less amount of time compared to currently used methods. Also, it is a safer and greener process as it produces very low amount of fines/dust and does not require the use of organic solvents. To conclude, outcome of the present research work can be utilized in industry for economic product development.

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1. Introduction

1.1. Introduction

Pelletization is generally referred to a size enlargement process that revamps fine powders or particles of bulk drugs with or without excipients into small, free flowing and roughly spherical units called as pellets. Pellets used in the pharmaceutical industry typically have a mean diameter ranging from 0.5 to 2 mm [1]. Pelletization is also referred as granulation or spheronization, and the spherical units obtained are called as granules, pellets, agglomerates or spheroids [2]. The general terms 'granulation' and 'pelletization' are sometimes used interchangeably, and no clear discrepancy is made between them. The concept of pellets was introduced in the pharmaceutical industry in 1949 when pharmaceutical researchers at Smith Kline & French (SKF) recognized the potential of candy seeds in developing controlled release formulation and instigated the development of tiny drug pellets that could be loaded into capsules [3]. Along with the pharmaceutical industry, applications of pellets are also found in the agribusiness (e.g. fertilizer, fish food, etc.) and in the polymer industry [4].

1.2. Advantages of pellets

In past few years, pellet formulation had undergone significant development owing to the better understanding of its advantages over single unit dosage form and flexibility found in their production. Benefits offered by pellets over single unit dosage form are well studied and documented by many researchers [1-6]. After administration, pellets disperse all over in the gastrointestinal (GI) tract which leads to augmented drug dissolution and absorption. Presence of food has a marginal effect on gastric emptying rates and overall transit time of pellets which minimize variation in inter-subject and intra-subject plasma profiles. Unlike, a single unit dosage form, pellets can avoid high local concentration of drug which may cause irritation or numbness to GI tract. With the usage of pellets having different release rates, peak plasma concentration level of drug can be lowered which significantly lessens potential adverse effects without compromising drug bioavailability. As controlled release formulations, pellets are less prone to dose dumping compared to the single unit dosage form.

Along with theses therapeutic advantages, pellets also offer series of benefits for manufacturing which have been amply documented in the literature [1, 4, 6, 7]. Pellets prevent the segregation of co-agglomerated components which helps to maintain content uniformity. It also causes marginal dust formation which offers improved process safety and minimal material loss. Pellets provide better flow properties, uniform packing,

narrow size distribution and less friable dosage form. Due to the low surface area to volume ratio, it provides an ideal shape for application of film coating. Pellets also facilitate simultaneous delivery of two or more chemically incompatible drug at the same or different sites in GI tract. Additionally, pellets offer flexibility in further modifications, such as a coating to obtain different release profiles, taste or odor masking. Also, it can be compressed into tablets.

1.3. Advantages of extrusion-spheronization technology

Pellets can be manufactured using powder layering [8-11], spray drying [12-15], spray congealing [16-18], fluidized bed technology [19-21], hot melt extrusion [22-26] and extrusion-spheronization. Currently, extrusion-spheronization is gaining immense popularity as it offers series of advantages over other technologies [3,4,7, 27-29]. Extrusion-spheronization is highly precise and operator independent technology. It is easy to operate and clean. It produces a higher yield and requires less time than powder layering, fluidized bed technology, etc. It produces very little amount of fine compared to powder layering technology which ensures improved process safety, minimal material loss and lower manufacturing cost. Pellets produced using extrusion-spheronization offer narrow size distribution and less friability. Pellets with very high drug loading (up to 90% w/w) can be manufactured using this technology. Furthermore, pellets manufactured with this technology exhibited superior controlled drug release profile when compared with other technique.

1.4. Extrusion-spheronization process and equipment

Extrusion-spheronization is a multi-stage process which involves four steps viz. mixing of ingredients and manufacturing of the wet mass (dry/wet mixing), shaping the wet mass into cylinders (extrusion), breaking up the extrudate and rounding of the particles into spheres (spheronization) and finally drying of the pellets. As these steps are interrelated, quality of end product is also significantly dependent on process and various process parameters involved [28]. Figure 1.1 illustrates the various steps of the process and process parameters involved in extrusion-spheronization.

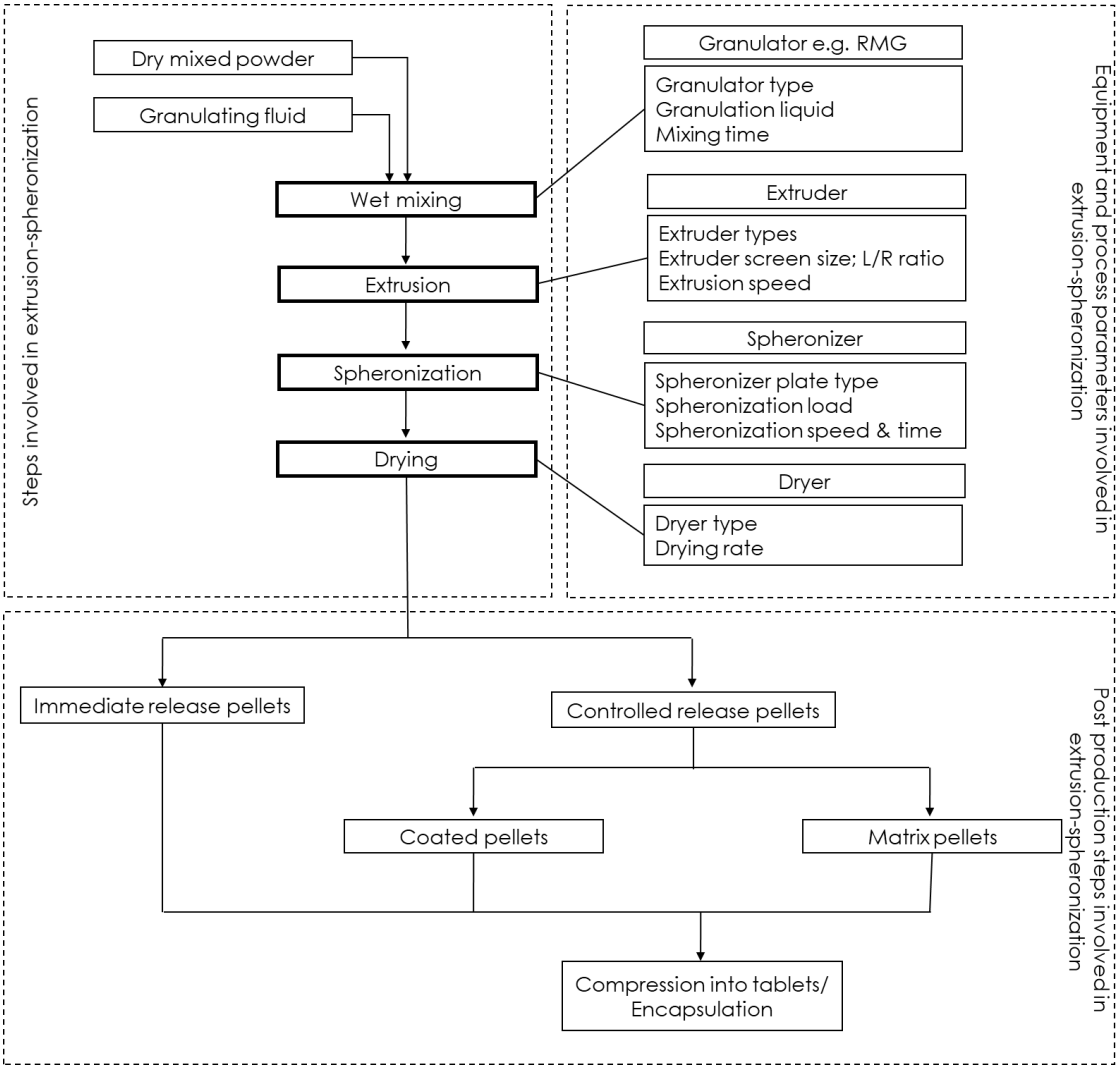


Figure 1.1. Various steps and process parameters involved in extrusion-spheronization technology

1.4.1. Mixing and wet granulation

First step of the extrusion-spheronization process is mixing or blending of dry powders. At this point, active pharmaceutical ingredient (API) is mixed with one or more excipients in a blender. Then, a suitable amount of binder solution or water is added to the dry powder blend at an appropriate rate to obtain a wet mass. Different types of granulator are used to perform these mixing processes. The most commonly used granulator is the planetary mixer [30-32], however, in various studies, use of a high shear mixer [33-35], sigma blade mixer [36] and a continuous granulator [37-38] has also been reported. There are two critical variables, viz. amount of granulation fluid that is added during wet mixing and uniform distribution of fluid throughout the powder mass, which must be properly controlled during the mixing process in order to obtain high quality pellets. Mixing speed, mixing time, and rate of granulation fluid addition are other

significant parameters that should also be considered for uniform distribution of fluid throughout the powder bed [39-44]. Further, evaporation of granulating fluid during this step can influence the extrusion behavior of wet mass. This can be a problem, especially with high shear granulator as they introduce a significant amount of energy during mixing and part of which transforms into heat. It should be controlled to a minimum. Cooling of granulation bowl might avoid this problem [4,45].

1.4.2. Extrusion

Extrusion is second step of the process which involves passing of wet mass through an orifice under pressure, to produce long noodle like rods, which are more commonly known as ‘extrudates. The extrudates break at similar lengths due to its own weight. Extrudates must have sufficient plasticity to deform however not so much to adhere to each other when collected or rolled in the spheronizer. Extrusion is performed using extruders. Extruders types, instrumentation, and process parameters have been painstakingly reviewed by many authors [4-7, 28, 45-50]. Extruders can be classified into three groups according to the feeding mechanism used in it. Figure 1.2 illustrates the classification of different types of extruders used in the pharmaceutical industry.

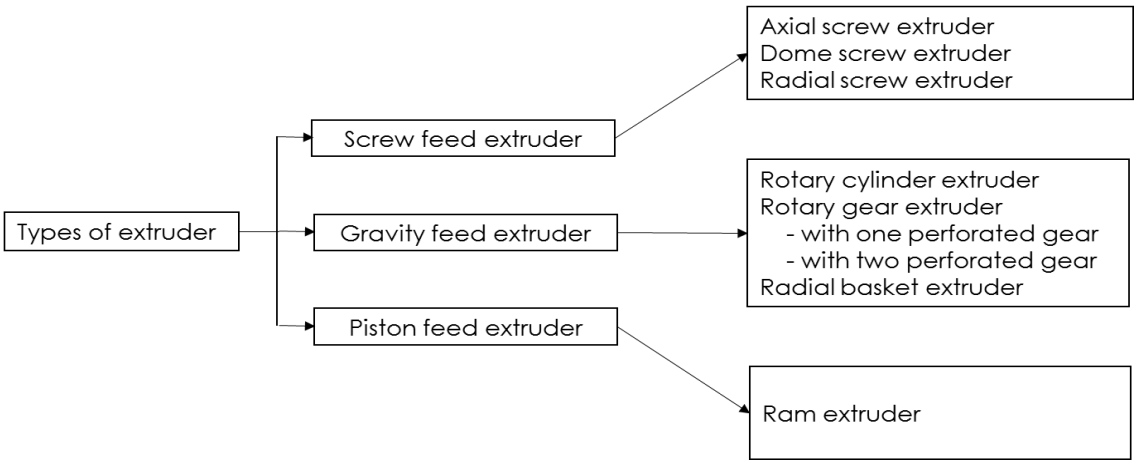


Figure 1.2. Classification of extruders used in extrusion-spheronization

1.4.2.1. Screw feed extruders

Screw feed extruders consist of one or two screws which feed the wet mass towards extrusion screen. In axial screw extruder [Fig. 1.3A], flat extrusion screen is positioned perpendicular to the axis of the screw. On the other hand, radial screw [Fig. 1.3B], extruder consists of an extrusion screen which is positioned parallel to the axis of the screw. Dome screw extruder [Fig. 1.3C] consist of a semicircular shaped screen which

is positioned perpendicular to the axis of screw similar to the axial screw extruder. Axial type extruder produces higher force due to longer die length. Due to this, it produces extrudates with a slightly higher density than other types of the screw extruder. However, this design has higher dead volume and also cause heat build-up during extrusion. Radial extruders have a higher yield than other screw extruders, but they produce less dense extrudates compared to those produced by the axial or dome extruders. Heat generated during the extrusion process is considerably less which allows the use of a radial extruder for the processing of thermolabile drug substances [4,7,28].

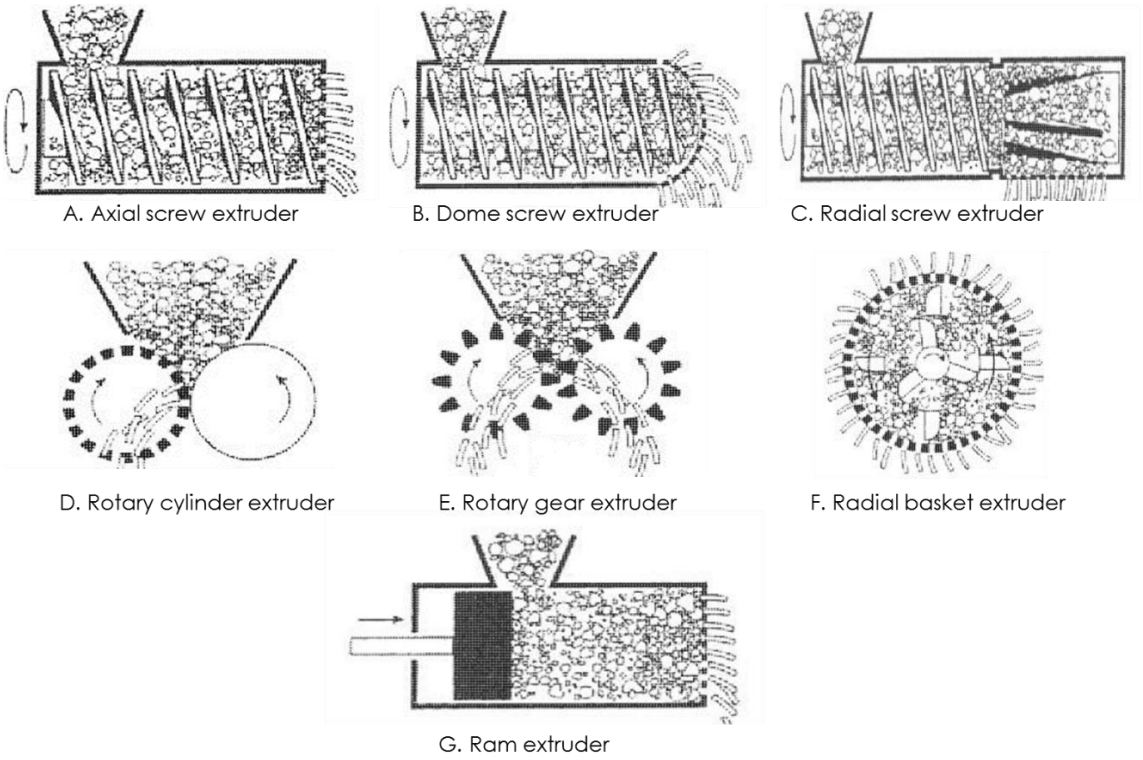


Figure 1.3. Schematic representation of extruder types used in extrusion-spheronization; Reproduced with permission from Mehta KA. et al. [46].

1.4.2.2. Gravity feed extruders

In gravity feed extruder, as the name indicates, wet mass transfers through the extruder using gravitational force. Three types of gravity feed extruders are currently available viz. rotary cylinder extruder, rotary gear extruder, and radial basket extruder. The rotary cylinder extruder [Fig. 1.3D] has one perforated and one solid roller. The perforated roller acts as die while the solid roller applies pressure and forces the wet mass through the die to yield extrudates. Rotary gear extruder [Fig. 1.3E] is equipped with two contra-rotating wheels in the form of gears. The base of the gear is perforated which acts as die. Rotary cylinder and rotary gear type of extruder have almost negligible dead volume.

However, cleaning is tough, and drilling cost is very high for these types of extruders. Radial-basket extruders are most widely used extruders in the pharmaceutical industry. Usually, radial-basket extruders produce less dense extrudates compared to those produced by the gear extruders [28].

1.4.2.3. Piston feed extruder

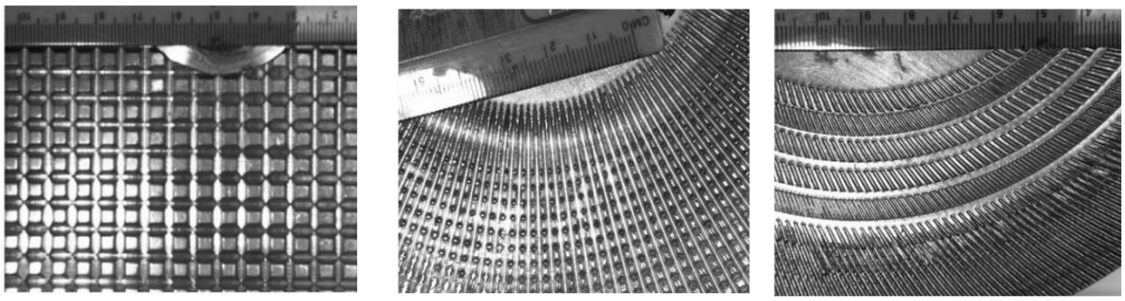
Piston feed extruder is also called as ram extruder [7,51-53]. Ram extruder [Fig. 1.3F] is considered to be the oldest type of extruder. These are generally used in plastic and rubber industry. Ram extruder consists of the piston which forces wet mass through the extrusion screen positioned at the end of the barrel. Ram extruders are primarily used in the development phase because it allows controlling critical parameters such as pressure, temperature, size and weight of the extrudates. Besides, they can also be used to evaluate the rheological properties of the wet mass.

The process variables of the extrusion process that influence quality of pellets include a type of extruder, extrusion speed, ratio of length and diameter of the extrusion screen holes (L/R ratio) and wet mass feeding rate to extruder [27, 54-62].

1.4.3. Spheronization

This step of extrusion-spheronization contains the placing of the extrudates inside spheronizer where extrudates are broken up into smaller cylinders with a length equal to their diameter and finally converts into spherical pellets. Spheronizer consists of a cylindrical bowl and a spinning plate, known as a friction plate, which spins at high speed at the bottom of a cylindrical bowl. The diameter of the spheronizer friction plate ranges from approximately 20 cm for laboratory-scale equipment to 100 cm for production-scale units. Depending on the diameter of the plate, the rotational speed of the friction plate varies from 100 to 2000 rpm. Usually, 2-10 min of spheronization is required to obtain optimum quality pellets [4-7, 27-29, 63-67].

The friction plate has a meticulously designed groove pattern on the processing surface. Three types of groove patterns exist viz. cross-hatch pattern, radial pattern and striated edge pattern [63]. Size of grooves pattern on plate varies from 0.8 mm pitch to 3 mm pitch. Usually, extrudates up to 0.8 mm in diameter are generally processed on 2 mm pitch plate whereas 3 mm pitch plate is used for extrudates up to 3 mm in diameter.



A. Cross-hatch pattern B. Radial pattern C. Striated edge pattern

Figure 1.4. Friction plate designs; Reproduced with permission from Michie H. et al. [63]

The most commonly used groove pattern for spheronizer friction plate is the square cross-hatched square design, where processing surface is covered with a grid of truncated pyramids [Figure 1.4A]. Friction plate with radial pattern exhibits grooves originate from the center like the spokes of a bicycle wheel [Figure 1.4B]. Plate with a radial pattern is used when gentler action is required on the material being spheronized. Use of striated edge plate [Figure 1.4C] can result in increased yield of pellets but it is harder to manufacture.

Also, the size and spacing of groove pattern of plates with a radial pattern changes with the size plate size which results into variation in frictional force effect. As a consequence of this, pellet properties have a variation with scale-up [27-28, 63]. Therefore, cross hatch patterned friction plate is more preferred for the spheronization process. Michie et al. used different spheronizer friction plate patterns (cross hatch, radial, striated edge pattern) to manufacture pellets using extrusion-spheronization technology to investigate whether the plate pattern affects the properties of pellets [63]. They observed that plate pattern affected yield, porosity, mechanical strength and release properties of the pellets.

Other process variables of spheronization process that influence the quality of pellets are spheronizer speed, spheronization time, and spheronization load. Between these variables, spheronizer speed and spheronization time are the most important factors that influence the quality of the pellets [63-70]. The spheronization speed influences size and size distribution of the pellets. A decreasing quantity of fines and a higher quantity of particles with an increased mean diameter was observed with faster spheronization speed [37-38, 71]. Kleinebudde et al. found that spheronization speed has an influence on the size but not on the porosity of pellets [72]. Umprayn et al. reported that an increase in

spheronization speed resulted in increased sphericity [73]. Ronowicz et al. documented that water content of extrudate, number of holes of extrusion screen, spheronization time, and spheronization speed are the important factors influencing pellet aspect ratio. The maximum spherical pellets are achieved by using a high spheronizer speed and longer time of spheronization [74]. Surface structure, hardness, friability, bulk and tapped density, flow properties of pellets are also influenced by a change in the spheronization speed [37-38, 70-71].

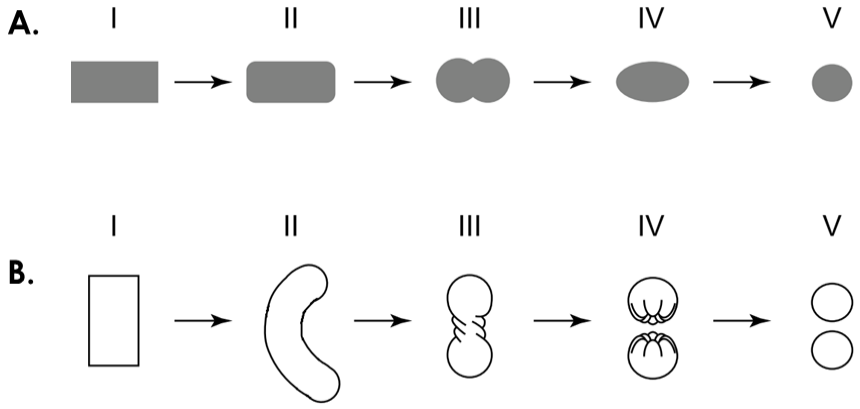


Figure 1.5. Pellet formation mechanism A. Rowe's mechanism - I. Cylinder II. Cylinder with rounded edges III. Dumbbell IV. Ellipse V. Sphere; B. Baert's mechanism - I. Cylinder II. Rope III. Dumbbell IV. Sphere with a cavity V. Sphere; Reproduced with permission from Gandhi R. et al. [5]

1.4.3.1. Pellet formation theories

Two principal mechanisms have been suggested for pellets formation. According to Rowe [75], only frictional force of the spheronizer plate is responsible for rounding of the cylindrical extrudates into spherical pellets. Rowe proposed that, during spheronization process, initially extrudates converts into rounded edges cylinders. Rounded edges cylinders then convert into dumbbells and elliptical shaped particles which finally convert into perfect spheres [Fig. 1.5A]. Another mechanism of pellet formation was proposed by Baert and Remon [44]. According to this theory, both frictional and rotational force of spheronizer plate are responsible for spherical pellets formation. In this mechanism, a twisting of cylinder occurs after the formation of cylinders with rounded edges. Finally, twisted cylinder breaks up into two distinct parts which have round and flat surface. Rotational and frictional forces involved in the spheronization process attributes to the folding edges of the flat side together like a

flower, forming the cavity observed in certain pellets. [Fig. 1.5B]. Usual time required for the spheronization of product is 2-10 min.

1.4.4. Drying

Final step of the extrusion-spheronization process is drying. Pellets can be dried at room temperature [76-79] or at elevated temperature using fluidized bed dryer [80-81], tray dyer [82-83], or using an oven [84]. Freeze dryer [85-86] and a microwave [87-88] oven have also been used for drying of pellets. Each drying technique has different drying temperature and drying rate. As a result, different drying techniques have different effects on properties of the end product. Bashaiwoldu et al. compared freeze drying, hot air oven drying, fluid-bed drying and desiccation with silica-gel. They reported that drying process has no effect on pellet shape. They also found that freeze-dried pellets have more porosity than pellets dried using other methods [89]. Many researchers have studied the effect of drying techniques on mechanical and drug release properties of pellets. Wlosnewski et al. compared three different drying techniques viz. hot air oven drying, freeze drying and microwave drying. They haven't observed any difference in release profile amongst microwave oven dried pellets and hot air oven pellets. Whereas, they found that freeze-dried pellets were porous which resulted in faster release [90]. Song et al. observed that freeze-drying maintained the shape and size of the pellets, however oven drying yielded pellets with roughened surface owing to the uneven shrinkage of wet pellets [91].

1.5. Evaluation of pellet quality

Quality of final pellets can be evaluated by estimating various parameters which includes pellet size and size distribution, pellet shape, surface morphology, surface area, hardness, friability, density, porosity, flow properties, disintegration time, in vitro drug release behavior and in vivo pharmacokinetic and pharmacodynamic performance.

1.5.1 Size and shape

Mean particle size and size distribution of pellets are critical parameters which mainly influence the drug content in pellets and drug release behavior of the formulation [92]. Pellets formulation should have narrow particle size distribution. Generally, sieve analysis is used to evaluate particle size distribution. However, image analysis techniques can also be used to determine particle size and size distribution [93]. Moisture content, amount and type of binder used, extrusion speed, spheronization speed and

spheronization time are the critical parameters which affect the particle size distribution [27-28].

Sphericity is one of the most important properties of pellets as it affects flow properties and coating performance of pellets. Shape of pellets can be represented by calculation of aspect ratio and pellet circulatory. Aspect ratio and pellet circulatory can be easily determined with the help of image analysis software using the digitalized image of pellets obtained through an optical microscope. Critical parameters which affect the shape of pellets are type and amount of spheronizing agent, spheronization speed and spheronization time [94].

1.5.2. Hardness and friability

Acceptable levels of hardness and friability are required to withstand mechanical stress during handling, shipping, storage and processing such as a coating, filling, etc. Friability is tendency of the pellets to chip off under mechanical stress during handling resulting in the formation of dust. It can be assessed using friabilators and tumblers. Different types of equipment (friabilators or tumblers) were used by various investigators for different time intervals and at a different speed in order to evaluate friability of pellets [7, 89, 95-96]. Steel and glass balls have also been used in friabilator to increase the mechanical stress [95, 97]. According to Reynolds [66], hardness of the pellets can be correlated with either friability or can be directly measure using texture analyzer or Khal hardness tester [98]. Number of process and formulation parameters can affect granule strength. Generally, larger granules possess higher strength than the smaller ones. The type and amount of binder used during wet mixing also affect the granule strength [7]. Other factors which affect hardness and friability are moisture content, type of excipients, type of friction plate used during spheronization, type drying technique and temperature [4,7,27-28].

1.5.3. Density, porosity and flow properties

Variation in pellet density can cause the batch to batch variation in coating level and capsule filling. Further, bulk and tap densities of pellets give an idea about the homogeneity of the particle size distribution [5]. Bulk density signifies the packing properties of particles and is highly influenced by the size of pellets and it can easily be determined by the measurement of volume. On the other hand, tap density of pellet can

be determined by using an automated tapper. Further, true density can be calculated using an air-comparison pycnometer or by the solvent-displacement method.

Porosity of pellets influences the adsorption, diffusion disintegration and dissolution of the drug from pellets [99]. It can be determined by gas adsorption method or mercury intrusion porosimetry technique. Gas adsorption method is used for measurement of pore diameter smaller than 2000 Å while mercury intrusion porosimetry technique is more suitable for pore diameter larger than 2000 Å [100-102]. Amount of granulation fluid, type of drying technique and drying rate are the most important parameters which affect the porosity of pellets [7].

Flow properties of pellets are evaluated to ensure a uniform filling of pellets in the capsules or in the die cavity for compression. Flow properties of pellets can be determined by a variety of static and dynamic methods. Static method includes determination of angle of repose, calculation of Carr's index and Hausner's ratio using bulk and tapped density data. On the other hand, dynamic flow method estimates the flow rate of the pellets through an appropriate orifice. Size and shape of the orifice used during dynamic flow study is selected based on the size of the capsule, or by the size of the die cavity, if the pellets are going to be compressed into tablets [28,103]. Pellets size and size distribution, shape, surface morphology and residual surface energy are the critical factors which affect flow properties of pellets [104].

1.5.4. Surface morphology

Surface texture and internal structure of pellets are the crucial aspects which influence the pellets properties such as flowability, friability, wettability and adhesion to various substrates during coating and drug delivery behavior. Morphology of the surface and internal structure (using cross-sections) of dried pellets can be assessed using scanning electron microscope (SEM) [105]. Image analysis of SEM micrographs is used to assimilate the quantitative information of fractal geometry and surface texture. Additionally, use of mercury intrusion porosimetry (MIP) enables the assessment of pore volume, mean pore size, and pore size distribution. Gomez-Carracedo et al. inferred that the texture analysis by means of SEM images and porosity analysis based on MIP data gives valuable information on the surface texture and the inner structure of pellets. This information enables one to quantify the influence of pellet composition on the surface roughness and pores, and its impact on the drug release [106]. The surface texture and

the inner structure of the pellets mainly depend on the amount of the drug, type of excipients and amount of the granulation liquid used, and other process parameters involved in spheronization and drying.

1.5.5. Disintegration and dissolution (in vitro drug release) study

Uncoated, coated, or compressed pellets should release the drug at desired rate. Hence, it is important to perform disintegration and dissolution study of pellets. Disintegration time is a particularly important evaluation parameter in case of immediate release pellets. Huyghebaert et al. [107] carried out disintegration test for pellets using the USP apparatus type 3 (reciprocating cylinder method) while few investigators [41] performed in a specially modified tablet disintegration tester.

In the development of drug delivery system, in vitro drug release study is one of the key parameters for the evaluation of the quality especially in modified release formulations. In vivo absorption of drug depends on the release of drug from the solid dosage form which enables us to correlate in vivo bioavailability of the drug with the data obtained from the in vitro dissolution studies of the formulation. The release of the drug from the pellets is primarily influenced by size, hardness, and porosity of the pellets. In vitro release of drug from pellets is determined by using a variety of dissolution tester such as USP apparatus type 1 (rotating basket), USP apparatus type 2 (paddle), etc. [108-109]. Amount and type of polymers, aqueous solubility of drug, physical state of drug in the matrix, drug load and the presence of additives such as surfactants also influence the drug release profiles of pellets [27].

1.6. Carbopol polymers

Carbopol polymers are high molecular weight, cross-linked, acrylic acid based polymers. In various pharmacopeias, Carbopol polymers also termed as carbomers, carboxyvinyl polymer, or carboxy polymethylene. Carbopol polymers were first patented in 1957 by B.F. Goodrich Company [110]. Since then, several extended release formulations, which involve Carbopol polymers matrices, have been reported. Currently, Lubrizol Advanced Materials Pvt. Ltd, is the largest manufacturer of Carbopol polymers in the world.

1.6.1. General properties of Carbopol polymers

Carbopol polymers are offered as white, fluffy and dry powders. The carboxyl groups present in the acrylic acid backbone of the polymer are accountable for many advantages of Carbopol polymers. These polymers have an average equivalent weight of 76 per

carboxyl group. The molecular weight range of Carbopol polymers is estimated to be from 740,000 to 4-5 million. At present, there is no method available to calculate the exact molecular weight of a three-dimensionally cross-linked polymer of this type. General structure of Carbopol can be represented with Figure 1.6.

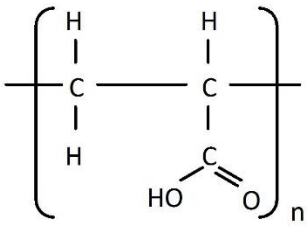


Figure 1.6. General structure of Carbopol polymers

These are flocculated powders averaging 2 to 7 microns in diameter. Carbopol polymers do not dissolve in water. On the other hand, when exposed to a pH environment above 4.0 - 6.0, these polymers swell in water up to 1,000 times their original volume to form a gel. The glass transition temperature of Carbopol polymer is 105 °C (221 °F) in powder form. However, it decreases drastically as the polymer comes into contact with water [111-114].

1.6.2. Types of Carbopol polymers

The major differences among the Carbopol polymers are associated with the cross-linker type and its density, and the presence of hydrophobic comonomers as summarized below [111].

1. **Carbopol homopolymers** are polymers of acrylic acid cross-linked with allyl sucrose or allyl pentaerythritol.
2. **Carbopol interpolymers** are carbomer homopolymers or copolymers that contain a block copolymer of polyethylene glycol and a long chain alkyl acid ester.
3. **Carbopol copolymers (Pemulen)** are polymers of acrylic acid and C10-C30 alkyl acrylate cross-linked with allyl pentaerythritol.
4. **Noveon polycarbophil** is a polymer of acrylic acid cross-linked with divinyl glycol.

Various grades of Carbopol polymer are available, and each grade has its distinctive significance in the preparation of pharmaceutical dosage form. Classification of currently available Carbopol polymers is given in Table 1.1.

Table 1.1. Classification of Carbopol polymers

Class	Carbopol grades
Carbopol homopolymers	Carbopol 971P NF, Carbopol 974P NF, Carbopol 71G NF, Carbopol 980 NF, Carbopol 981 NF and Carbopol 5984 EP
Carbopol interpolymers	Carbopol ETD 2020 NF and Carbopol Ultrez 10 NF
Carbopol copolymers	Pemulen TR-1 NF and Pemulen TR-2 NF
Noveon polycarbophil	Noveon AA-1 USP

1.6.3. Carbopol safety and regulatory guidelines

Initially, benzene was used as a solvent during the manufacturing process of Carbopol polymers. Due to regulatory restrictions on the use of benzene in pharmaceutical formulations, in the 1980s, Lubrizol started manufacturing newer grades of Carbopol polymer with new processes using ethyl acetate and ethyl acetate/cyclohexane co-solvents to enhance product safety. It is now recommended that benzene free Carbopol polymer grades should be used for all new drug development projects. Furthermore, benzene polymerized Carbopol polymers should be substituted with a non-benzene polymerized Carbopols in an already established pharmaceutical formulation. Lubrizol recommended substitutes for the benzene grade Carbopol products based on viscosity criteria have been summarized in Table 1.2. It should be taken into account that these substitute non-benzene polymers are not identical and their performance in a formulation may be different.

Table 1.2. Recommended substitute for benzene grade polymer

Benzene grade polymer	Recommended non-benzene grade polymer
Carbopol 934 NF	Carbopol 5984 EP and Carbopol Ultrez 10 NF
Carbopol 934P NF	Carbopol 974P NF
Carbopol 940 NF	Carbopol 980 NF and Carbopol Ultrez 10 NF
Carbopol 941 NF	Carbopol 71G NF, Carbopol 971P NF and Carbopol 981NF
Carbopol 1342 NF	Pemulen TR-1 NF and Pemulen TR-2 NF

1.6.4. Application of Carbopol polymers

Carbopols are highly efficient controlled release agent and in many studies demonstrated slower release at lower use level than other commercially available release retarding polymers such as hydroxypropyl methylcellulose (HPMC) thus enabling cost saving and smaller size of the solid dosage form such as a tablet. Carbopol 971P, Carbopol 71G, and

Carbopol 974P are most widely used excipients in controlled release tablet formulations [115-118]. Carbopols also have excellent bioadhesive properties. Noveon AA-1 USP polycarbophil is one of the most commonly used bioadhesive agents. Many oral and topical bioadhesive formulation have been formulated with Carbopol polymers [120-122]. Carbopols are excellent rheology modifier. It can be used at very low concentrations (less than 1%) to produce a wide range of viscosities and flow properties in lotions, creams and gels, transdermal gel reservoirs, and in oral suspensions [123-126]. Further, they are also utilized as emulsifying agents and can be used for topical oil in water emulsion, even at elevated temperature, with essentially no need for surfactants. Apart from that, they can be used as a suspending agent in the topical or oral formulation.

1.7. Problem definition and research objectives

The pharmaceutical applications of the extrusion-spheronization for the manufacturing of pellets were first documented in the early 1970s [64-66]. It has gained importance in last decade as primary choice for pellet preparation. Many researchers have worked on this technology and explored various formulation variables such as type and amount of granulation liquid, drug characters and form (free base, salts, etc.), type and amount of matrix formers and other excipients such as pore formers and electrolytes, which have a profound effect on the properties of pellets developed with extrusion-spheronization technology. Additionally, various process variables affecting the final pellet quality such as type of mixing equipment, mixing time, screen aperture diameter, screen pressure, extruder type and speed, extrusion temperature, type of friction plate, spheronization speed, spheronization time and load, drying technique and temperature have been well studied and documented [4, 7, 28].

Along with various formulation and process parameters, many researchers have explored different pelletization aids. Microcrystalline cellulose (MCC) especially Avicel PH101 is considered as the gold standard for spheronization aid. However, it has few limitations such as incompatibility with a number of drugs, drug adsorption on the surface, lack of disintegration, etc. Thus, many researchers have explored alternative spheronization aids such as powdered cellulose, starch derivatives, chitosan, κ-Carrageenan, pectinic acid, HPMC, hydroxyethyl cellulose (HEC), cross-linked polyvinylpyrrolidone (PVP), glyceryl monostearate, polyethylene oxide, alginates, β cyclodextrin, ethyl cellulose, glucose, mannitol, sorbitol, etc. [29, 40, 127-130]. Recently, few researchers explored the possibility of incorporating alternative forms of a drug such as self-emulsifying

systems, solid dispersions, etc. into pellets to achieve enhanced solubility along with a controlled release for better therapy [131-133].

Despite the extensive research and use of the extrusion-spheronization process in pharmaceutical formulations, the use of Carbopol polymers in this technology has not been fully studied and explored. The primary reason behind that is Carbopols become tacky when wetted, which causes handling difficulties. A literature review revealed that few studies were carried out for the development of Carbopol pellets using extrusion-spheronization [134-141]. In all studies, they have used strong electrolytes especially calcium chloride in granulation fluid to reduce the tackiness of Carbopol polymers. Unfortunately, the addition of strong electrolytes also resulted in diminished release retarding property and bioadhesive nature of the Carbopol polymer [134]. The carboxyl groups provided by the acrylic acid backbone of the Carbopol polymer, hydrate in the presence of water and tend to dissociate in an aqueous system. This dissociation results in negative charge on the polymer backbone which then repel each other to cause dramatic polymer expansion. These swelling and hydrophilic characteristics are responsible for release retarding and other beneficial properties of Carbopol. The addition of electrolytes neutralized negative charge on polymers backbone and resulted in diminishing major beneficial properties of Carbopol polymers.

To the best of our knowledge, various formulation and process parameters involved in extrusion-spheronization for the development of Carbopol pellets, without using any strong electrolytes, have not been explored fully. Furthermore, the addition of Carbopols in wet mass resulted in extrudates with higher plasticity which are difficult to break during spheronization process. Thus, there is a need to develop an alternative process for development of Carbopol pellets using extrusion-spheronization technology. Also, there is a need to study the effect of various formulation and process parameters and identify the crucial parameters having a significant effect on the pellet quality. Additionally, the process should be optimized to obtain pellets having good sphericity with a minimal amount of spheronization time.

Currently, the majority of extended release pellets are manufactured using powder layering method and fluidized bed processor. Owing to outstanding release retarding properties of Carbopol, use of these polymers may help to use extrusion-spheronization technology to prepare matrix pellets in single step with similar extended release profile.

Powder layering method and fluidized bed processor are multistage processes which require a significantly higher amount of time than extrusion-spheronization. Also, both these methods require a large amount of organic solvent while extrusion-spheronization only requires water. Unlike both these methods, extrusion-spheronization produces very little amount of fine and higher amount of yield. Powder layering method is an operator-dependent process which requires a skilled operator. Extrusion-spheronization is a single step, rapid, precise method and does not require a skilled operator. Also, pellets manufactured with this technology are dense and hard, making them more suitable for extended release formulations.

Furthermore, Carbopols are widely compatible with most active pharmaceutical ingredients and commonly used excipients in oral solid dosage forms. They can be combined with other matrix forming excipients, if needed, to achieve the desired release profile. Carbopols may impart additional functions such as taste masking, binding, bioadhesion, etc. and it may also increase bioavailability. Therefore, Carbopol can be used in extrusion-spheronization technology for designing better and controlled drug delivery systems with lower cost. Owing to these therapeutic and manufacturing advantages, the primary objective of the present research work was to develop and evaluate pellet formulations with extrusion-spheronization technology using Carbopol polymers. To achieve this broad objective following specific objectives were laid down.

- Design and development of Carbopol based pellets for selected drugs using extrusion-spheronization technology without the addition of any electrolytes.
- Optimization of various formulation and process parameters involved in extrusion-spheronization to achieve pellets with desired characteristics
- Characterization of optimized pellets formulation for size, shape, yield, hardness, friability, flow properties, drug content, etc.
- Evaluation of in vitro drug release performance of the designed pellet formulations
- Pharmacokinetics studies of the select pellet formulations in rats

The proposed research work required analytical methods for preformulation studies, formulation development and stability studies, and for pharmacokinetic study. Therefore, it was planned to develop and validate a simple, rapid and sensitive ultraviolet (UV) spectroscopic method, stability indicating high performance liquid chromatographic (HPLC) method and bioanalytical (HPLC-UV) method to estimate venlafaxine and domperidone in bulk drug and different matrices.

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2. Drug Profile

2.1. Domperidone

2.1.1. Introduction

Domperidone, benzimidazole derivative (Figure 2.1), is a peripheral dopamine antagonist with antiemetic and gastroprokinetic properties [1]. Its action is similar to that of metoclopramide hydrochloride. Its inability to cross blood-brain barrier attributed to fewer neurological adverse effects than metoclopramide [2]. It thus offers superior safety profile for long-term oral administration in the recommended doses. It has been indicated for the management of dyspepsia, nausea, vomiting and gastro-oesophageal reflux disease (GERD).

2.1.2. Drug information

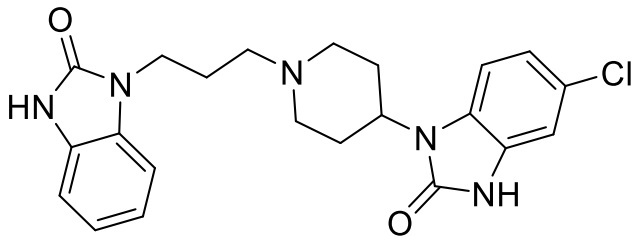

Figure 2.1. Structure of domperidone

Drug name	: Domperidone
Category	: Antiemetic, gastroprokinetic
IUPAC name	: 6-chloro-3-[1-[3-(2-oxo-3H-benzimidazol-1-yl)propyl]-piperidin-4-yl]-1H -benzimidazol-2-one
CAS number	: 57808-66-9
Chemical formula	: $C_{22}H_{24}ClN_5O_2$
Molecular weight	: 425.911
Melting point	: 242.5 ºC
logP	: 3.9
pKa	: 7.9
Solubility	: It is very slightly soluble in water (0.987 mg l^{-1}); slightly soluble in methanol; sparingly soluble in dimethyl formamide; and very slightly soluble in alcohol
BCS class	: Class II

2.1.3. Mechanism of action

Mechanism of action and other pharmacological aspects of domperidone have been systematically reviewed [1-7]. It is dopamine antagonist which acts on D2 & D3 receptors. Its antiemetic effect attributed to its action on both central dopamine receptors located in chemoreceptor trigger zone and peripheral dopamine receptors found in the gastro-oesophageal and gastroduodenal junctions. Gastroprokinetic effect is related to its action on the peripheral receptor. Domperidone assists gastric emptying and reduces small bowel transit time by lowering esophageal sphincter pressure and by enhancing gastric and esophageal peristalsis. Domperidone may also inhibit cholinesterase activity.

2.1.4. Pharmacokinetics

Pharmacokinetics and other pharmacological parameters have been explored and reported [8].

Absorption: In fasting subject, it absorbed rapidly with oral administration and attended peak plasma concentration within 30 to 60 min. Extensive metabolism of domperidone in liver and gut wall lead to its lower bioavailability (approximately 15%). Slight delay in peak time and somewhat increased area under the curve (AUC) was observed when the oral medicine taken after the meal.

Distribution: It has 90 to 93% of plasma protein binding. Distribution studies with the radiolabeled drug in animals have shown wide tissue distribution, but low brain concentration. Higher molecular weight and lower lipid solubility of domperidone resulted in its minimal penetration through the blood-brain barrier. A small amount of drug crossed placenta in rats.

Metabolism: It is extensively metabolized in liver and gut wall by N-dealkylation and hydroxylation. Through hydroxylation domperidone is metabolized to hydroxy-domperidone and through oxidative N-dealkylation, it is metabolized to 2, 3-dihydro-2-oxo-1-H-benzimidazol-1-propionic acid.

Elimination: Urinary and fecal excretion is the major mode of elimination. Approximately 66% of drug metabolites excreted through fecal excretion while urinary excretion amounts to 31%. Only small fraction of drug excreted as an unchanged drug (1% of urinary and 10% of fecal excretion). After single oral dose administration, plasma

half-life was found to be 7 to 9 hr in healthy subjects. Plasma half-life is extended to several hours in the patients with severe renal insufficiency.

2.1.5. Therapeutic use

Nausea and vomiting: Domperidone has antiemetic activity, and it is beneficial for the treatment of nausea and vomiting induced by a drug or other sources. [2, 3].

Gastro-oesophageal reflux disease (GERD): Domperidone is used to treat symptomatic GERD. Treatment for GERD with a combination of omeprazole and domperidone is more effective than omeprazole alone [9]. However, effectiveness of domperidone alone is not well established [10].

Gastroparesis: It is a medical condition characterized by weakness of gastric muscle which is attributed to delay in emptying of the stomach. Nausea, vomiting, abdominal pain, bloating, early and fullness after eating are major symptoms. Domperidone is useful in gastroparesis due to its muscle stimulant activity [11, 12]. However, increased rate of gastric emptying induced by domperidone and gastroprokinetic drugs does not always correlate (equate) well with relief of symptoms [13].

Functional dyspepsia: Domperidone is beneficial for the treatment of functional dyspepsia associated with lower gastrointestinal motility [14-16].

Lactation: Galactagogue activity of domperidone is ascribed to its anti-dopaminergic property. Dopamine inhibits secretion of prolactin. Domperidone assists prolactin release which is essential for lactation by blocking dopamine release [17-19]. It is thought to be effective and safe to use for lactation [20, 21].

Parkinson's disease (PD): Domperidone is used to treat gastric symptoms in a patient with PD. It is safe as it does not cross blood-brain barrier [22, 23].

2.1.6. Dosage and administration

Adults: 10 mg of drug three to four times daily. The maximum daily dose is 80 mg. Safety and efficacy of domperidone use studies have been established up to six months only [1, 8, 16].

In patients with severe renal insufficiency: Increase in elimination half-life of domperidone is reported in a patient with renal insufficiency. Dose adjustment is not required for single acute administration since the minute amount of unchanged drug is

excreted via the kidneys. However, the frequency of dosing should be reduced to once or twice daily for repeated administration. Depending on the severity of the impairment dose may need to be reduced for repeated administration [8].

Food: It is recommended that domperidone should be taken before meals. However, if taken after meals absorption of the drug is somewhat delayed.

2.1.7. Adverse effects

Adverse effects witnessed during long-term studies with domperidone are possibly related to increases in serum prolactin. These effects include swelling or tenderness of the breasts, breast secretion and lactation, gynecomastia, irregular menses, amenorrhea and a decrease or loss of libido. These effects observed in patients who received up to 120 mg per day in four divided doses [1, 8]. Dry mouth and headache are most common side effects of domperidone [8]. The extrapyramidal disorder occurs very rarely and primarily observed in young children. Convulsion, agitation and other central nervous system-related adverse effects are also reported especially in infants and children [2, 8].

2.1.8. Drug interaction

The primary metabolic pathway of domperidone is enzymatic degradation through CYP3A4. In vitro and human data shows increased plasma levels of domperidone with concomitant use of drugs that significantly inhibit the CYP3A4 enzyme. Examples of CYP3A4 inhibitors include azole antifungals such as itraconazole, fluconazole, voriconazole, and ketoconazole; HIV protease inhibitors, such as atazanavir, amprenavir, indinavir, fosamprenavir, nelfinavir, saquinavir, and ritonavir; macrolide antibiotics, such as erythromycin and clarithromycin; calcium antagonists, such as diltiazem and verapamil [24]. Domperidone increases movement in the digestive tract which may affect the absorption of other medications, in particular with monoamine oxidase inhibitor (e.g., furazolidone, phenelzine, selegiline, tranylcypromine).

2.1.9. Commercially available formulations

Domperidone was developed by Janseen-Cilag, now part of Johnson & Johnson Ltd., and marketed under the proprietary name of Motilium® (10 mg tablets) for the treatment of nausea, vomiting, and symptoms associated with idiopathic or diabetic gastroparesis. Domperidone is approved in India, Uinted Kindom, and many other countries and can now be officially prescribed to patients in the United States through an expanded access investigational new drug application for the treatment of gastroesophageal reflux disease

with upper gastrointestinal symptoms, gastroparesis, and chronic constipation. Domperidone is available in single dosage form or fixed-dose combination with proton pump inhibitors. Domperidone is available in the form of tablets, fast dissolving tablets, pellets, suspensions, and suppositories. Currently, in India, Cezvom® 10 mg tablets (Sandoz Ltd.), Domperi DT® 10 mg dispersible tablets (IPCA Ltd.), Tridom DT® 10 mg dispersible tablets (Zydus Ltd.), Domperon® 1 mg ml^{-1} suspension (Cadila Healthcare Ltd), etc. brands for commercial formulations of domperidone are available.

2.2. Venlafaxine

2.2.1. Introduction

Venlafaxine, bicyclic phenylethylamine derivative (Figure 2.2), is an antidepressant of the serotonin-norepinephrine reuptake inhibitor (SNRI) class. [25]. It is a unique anti-depressant which structurally differs from all other currently available antidepressants. It is metabolized into O-desmethyl venlafaxine (ODV) which is a potential antidepressant [26].

2.2.2. Drug information

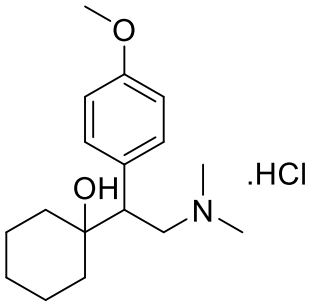

Figure 2.2. Structure of venlafaxine hydrochloride

Drug name	: Venlafaxine hydrochloride
Category	: Anti-depressant
Class	: Serotonin-norepinephrine reuptake inhibitor
IUPAC name	: 1-[2-(dimethylamino)-1-(4 methoxyphenyl)ethyl]cyclohexan-1-ol
CAS number	: 93413-69-5
Chemical formula	: $C_{17}H_{27}NO_2$ HCl
Molecular weight	: 313.87
Melting point	: 215 - 217 ºC
logP	: 2.48
pKa	: 8.91 (strongest basic); 14.42 (strongest acidic)
Solubility	: 572 mg ml^{-1} in water; also, soluble in ethanol, dimethyl sulfoxide (25 mg ml^{-1}), dimethylformamide (20 mg ml^{-1})
BCS class	: Class I

2.2.3. Mechanism of action

Mechanism of action and other pharmacological aspects of venlafaxine have been explored painstakingly [25-31]. However, the exact mechanism of action of venlafaxine is not clearly established so far. The antidepressant action of venlafaxine in humans is thought to be allied with its potentiation of neurotransmitter activity in the central nervous system. Venlafaxine and ODV, prevent the reuptake of both serotonin and norepinephrine with a potency greater for the 5-HT than for the NE reuptake process. Additionally, they weakly inhibit dopamine reuptake. Unlike tricyclic anti-depressants, they do not inhibit monoamine oxidase and neither exhibit affinity for brain muscarinic, cholinergic, histaminergic, or alpha-adrenergic receptors. Hence, it is devoid of the anticholinergic, sedative, and cardiovascular effect like a tricyclic antidepressant.

2.2.4. Pharmacokinetics

Detailed pharmacokinetic studies have been carried out by Wyeth Pharmaceuticals Inc., USA [32].

Absorption: Absorption of venlafaxine is high; at least 92% of the drug is absorbed after single dose administered orally. It undergoes extensive metabolism in the liver and ODV is the only major active metabolite. As a result, the absolute bioavailability of venlafaxine is about 45%. Absorption of venlafaxine or its active metabolite is not affected by food, and they exhibit linear kinetics over the dose range of 75 to 450 mg per day [33-35].

Distribution: Venlafaxine has 27 ± 2% of plasma protein binding while its active metabolite, ODV, has 30 ± 12% of plasma protein binding. The volume of distribution reported for venlafaxine and its active metabolite is $7.5 \pm 3.7\ l\ kg^{-1}$ and $5.7 \pm 1.8\ l\ kg^{-1}$ respectively [36].

Metabolism: Following absorption, venlafaxine undergoes extensive presystemic metabolism in the liver, primarily to ODV which is catalyzed by CYP2D6. It is also metabolized to N-desmethyl venlafaxine (NDV), N,O-di-desmethyl venlafaxine (DDV), and other minor metabolites. The amount of ODV formed is 56% while 16% of DDV and 1% of NDV are also produced [32-36].

Elimination: Urinary excretion is the primary mode of elimination. Approximately 87% of drug metabolites excreted through urinary excretion within 48 hr which contains

unchanged venlafaxine (5%), unconjugated ODV (29%), conjugated ODV (26%), and other minor inactive metabolites (27%) [32-35].

2.2.5. Therapeutic use

Venlafaxine is mainly used for the treatment of depression, general anxiety disorder, social phobia, panic disorder and vasomotor symptoms [25-27]. Studies have revealed the effectiveness of venlafaxine for conditions such as diabetic neuropathy and migraine prophylaxis, though it is not a primary choice [37]. Some studies have suggested the efficacy of venlafaxine in the treatment of attention deficit-hyperactivity disorder [38].

2.2.6. Dosage and administration

Adults: Depending on type and severity of disorder dose may vary over the range of 75 to 375 mg per day.

In patients with severe renal insufficiency: Plasma half-life is extended in the patients with severe renal insufficiency and liver disease. For that reason dose adjustment is necessary with these patients [34].

2.2.7. Adverse effects

Compared to older tricyclic antidepressants, venlafaxine has lower toxicity profile [39-40]. However, a higher dose can lead to serious side effects. Further studies show that venlafaxine has higher toxicity profile compared to selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine and paroxetine [41]. Commonly reported side effects of venlafaxine include anorgasmia, constipation, asthenia, drowsiness, dizziness, insomnia, nervousness, nausea, headache, decreased appetite, delayed ejaculation, diaphoresis, and xerostomia.

2.2.8. Commercially available formulations

Wyeth Pharmaceuticals Inc., first introduced venlafaxine in 1993, now marketed by Pfizer Inc., under proprietary name of Effexor® (immediate and sustained release tablets) for the treatment of the major depressive disorder, generalized anxiety disorder, panic disorder and social phobia. Venlafaxine is available in the form of immediate release tablets, sustained release tablets, and capsules. Currently, in India, Dalium® 37.5/75 mg tablets (Piramal Healthcare Ltd.), Envelaf® 75/150 mg capsules (Alkem Laboratories Ltd.), Venla XR® 37.5/75 mg extended release capsule, etc. brands for commercial formulations of venlafaxine are available.

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3. Analytical and Bioanalytical Methods

3.1. Introduction

The key purpose of any pharmaceutical formulation development is to design, develop and validate the process which should consistently yield a pharmaceutical formulation of a predetermined quality. In order to assess the quality of formulation, suitable analytical methods are essential at each stage of formulation development. For instance, suitable analytical methods are essential for the estimation of the drug in bulk, formulations, in vitro drug release samples, stability studies, in vivo pharmacokinetic studies and bioavailability studies. As per the need of study, analytical methods with different sensitivity can be developed using ultraviolet-visible (UV) spectrophotometer, spectrofluorometer, gas chromatography (GC), high performance thin layer chromatography (HPTLC), high performance liquid chromatography (HPLC), high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS), etc.

As analytical methods have prime importance in any product development process, it is necessary to ensure that the employed analytical method should provide accurate and precise data [1-2]. Hence, it is obligatory to develop and validate a simple, sensitive and accurate analytical method [3-5]. Drug control and regulatory agencies across the globe have recognized the importance of analytical sciences in product design and development process and have released comprehensive guidelines on the validation requirement in recent years [6-9]. Even though analytical method validation requirements depend on the nature of the analyte and analytical instrument, it generally comprises the specificity and selectivity, sensitivity, linearity and range, accuracy and precision, stability, etc. [10]. Also, most of the regulatory agencies emphasize on the use of stability indicating methods for the establishing stability profile of the drug substance and drug product [11]. Requirements also demand a precise qualitative and/or qualitative assessment of impurities and degradation products. Such analytical methods are also useful in preformulation studies [12]. Thus, well developed and validated analytical methods become a pre-requisite for successful formulation development.

HPLC and LC-MS methods require sophisticated instrument and complex procedures. These methods are also time consuming and costlier. Thus, for routine in vitro analysis, UV spectroscopic methods are generally preferred. There are few UV-spectroscopic methods reported for domperidone and venlafaxine hydrochloride [13-18]. However, use of organic solvents, complex procedure and need of additional dilution steps for in vitro release study are the major limitations of these reported UV spectroscopic methods.

Thus, there is a need for a simple, rapid and cost-effective UV methods for routine analysis in present research work.

Present research also requires estimation of domperidone and venlafaxine hydrochloride from various preformulation and stability study samples. As per ICH Q1 guidelines, fully validated and stability indicating method should be used for stability studies [19]. A stability indicating method is defined as an analytical method that accurately determines the active ingredients without interference from degradation products of active pharmaceutical ingredients (API) and other formulation excipients, process impurities or other potential reagent impurities. Few HPLC methods dealing with estimation of domperidone in bulk and pharmaceutical formulations are reported but these methods are moderately sensitive and many of these methods require a very high amount of organic solvent [20-24]. Likewise, for determining venlafaxine hydrochloride in bulk material and pharmaceutical formulations, very limited stability indicating methods using HPLC have been reported [25-26]. Reported stability indicating HPLC methods are not sensitive up to nanogram concentration and have not been validated thoroughly as per the ICH guidelines. Thus, there is a need for a much more sensitive, green and cost-effective stability indicating LC method for the estimation of both drugs.

It is always preferable to obtain successful findings in animal models before extending the studies to human. Therefore, the availability of simple analytical methods in rat plasma for the estimation of the drug is always beneficial for pharmacokinetic studies of drug and its novel formulations. An extensive literature survey has revealed that several methods are available including radioimmunoassay [27-30], mass spectroscopic [31-33], electron capture detection [34] and HPLC with fluorescence detection [35-36] for estimation of domperidone in biological matrices such as rat plasma, human plasma, brain, etc. Among these, HPLC methods are the most convenient as they require minimal sample preparation and lower cost compared to LC-MS. However, currently, HPLC with UV detection method is not available for estimation of domperidone in rat plasma. Thus, it was planned to develop more sensitive and simple HPLC-UV method for estimation of domperidone in rat plasma.

So, objective of the present study was to develop a simple, sensitive and economical UV spectroscopic methods for the estimation of domperidone and venlafaxine hydrochloride for routine analysis and in vitro release studies. Also, it was planned to develop a simple

and sensitive stability indicating HPLC methods in order to evaluate physicochemical properties, compatibility with excipients and stability studies. Moreover, for evaluation of formulations in an animal model, a selective and sensitive bioanalytical method for determination of domperidone in rat plasma was also developed. In addition, in-house developed methods were validated using appropriate statistical tests as per the standard regulatory guidelines set for analytical and bioanalytical methods. Furthermore, methods were successfully assessed for the determination of domperidone and venlafaxine hydrochloride in respective matrices.

Part I. Analytical and bioanalytical methods of domperidone

A. UV spectroscopic method for the estimation of domperidone

3.2. Experimental protocol

3.2.1. Reagents and chemicals

Domperidone (assay ≥ 99.78%) was purchased from Lee Pharma Ltd., India. A marketed formulation containing 10 mg of domperidone (Domperi DT, IPCA Laboratories Ltd., India) was purchased from the local Indian market. All other chemicals and reagents used were of analytical grade and purchased from SD Fine-Chem Ltd., India. Ultrapure water was obtained using Millipore purification assembly (Mill-Q Plus, Millipore Corporation, USA).

3.2.2. Instruments

Spectroscopic measurements were performed using a double-beam UV-Vis-NIR spectrophotometer (Shimadzu Corporation, Japan; model: UV3600) connected to a computer loaded with UVProbe software (Version 2.42). For intermediate precision study, a double-beam UV-Vis-NIR spectrophotometer (Shimadzu Corporation, Japan; model: UV1800) connected to computer loaded with UVProbe software (Version 2.42) was used. Both the instruments have an automatic wavelength accuracy of 0.1 nm and matched quartz cells with 10 mm path length.

3.2.3. Analytical method development

In the preliminary study, various aqueous buffers and organic solvents were screened to develop a suitable UV-spectrophotometric method for the analysis of domperidone in formulations. Media was selected based on solubility of the drug, good sensitivity, ease of sample preparation, economy of the method and its applicability. Drug solutions in

the selected media were scanned in the range of 200-400 nm wavelengths for selecting the wavelength of analysis.

3.2.4. Calibration standards

100 µg ml^{-1} stock solution of domperidone was prepared by dissolving 10 mg of the drug in 100 ml of selected media. Different concentrations were prepared from stock solution by transferring aliquots of stock solutions into a series of 10 ml standard volumetric flasks and volumes were made with selected media. Six different concentrations of domperidone in selected media were prepared in the range of 5-35 µg ml^{-1} to develop calibration curve.

3.2.5. Analytical method validation

The developed method was validated in terms of specificity, selectivity, linearity, range, precision, accuracy, limit of detection, limit of quantification, and robustness as per USP and ICH guidelines [6-7].

Apparent molar absorptivity and Sandell's sensitivity were calculated according to the standard formula. Specificity and selectivity of the proposed method were established by preparing drug solution in selected media along with and without common formulation excipients such as microcrystalline cellulose, di-calcium phosphate, starch, Carbopol 971, Carbopol 974, etc. All the samples were scanned from 200-400 nm at a speed of 200 nm min^{-1} and recorded spectra were checked for changes in the absorbance at respective wavelength. In a separate study, drug concentration of 20 µg ml^{-1} was prepared for pure drug and marketed formulation in selected media and analyzed (N = 5). Paired t-test at 95% level of significance was used to compare the means of absorbance.

To establish linearity of the proposed method, nine separate series of solutions of the drug concentrations (5-35 µg ml^{-1}) were prepared from the stock solutions and analyzed. Least square regression analysis was performed on obtained data. ANOVA test (one-way) at 95% significance level was performed on the absorbance values observed for each drug concentration during the replicate measurement of the standard solutions.

To determine the accuracy of the proposed method, different quality control (QC) levels of drug concentration [lower quality control (LQC), medium quality control (MQC) and higher quality control (HQC)] were prepared from independent stock solution and analyzed. Accuracy was assessed as the mean percentage recovery and percentage bias

[% Bias = 100 × (Predicted concentration – Nominal concentration)/Nominal concentration] for each level of the QC standards. Further, accuracy was validated with recovery studies using standard addition method. In this method, different concentrations of the pure drug were added to a known pre-analyzed formulation sample and analyzed using the proposed method to check the recovery. The percentage analytical recovery of the added pure drug was calculated as: % analytical recovery = $[(C_T – C_F)/C_A] \times 100$, where C_T is the total drug concentration measured after standard addition, C_F is the drug concentration in the formulation sample, C_A is the drug concentration added to the formulation sample.

For precision study of proposed method, intra-day and inter-day precision studies were carried out by estimating the responses of three QC standards, three times on the same day and on three different days respectively. Further, one set of QC standards were re-analyzed using another UV spectrophotometer to determine inter-instrument precision. The precision was expressed as percentage relative standard deviations (%RSD) of the predicted concentrations from the regression equation of proposed method.

The limit of detection (LOD) is defined as the lowest detectable concentration and limit of quantification (LOQ) is defined as lowest quantifiable concentration. LOD and LOQ were calculated as 3.3 σ/s and 10 σ/s respectively, where 'σ' is standard deviation (SD) of intercept and 's' is slope of the calibration curve.

To determine the robustness of the developed method, all three QC standards were analyzed in selected media with a change in pH by ± 0.2 unit and mean percentage recovery was calculated. Also, as part of robustness study, stability of domperidone in the selected solvent medium was evaluated using QC samples at room temperature. QC samples were analyzed at different time intervals (24 hr, 48 hr and 72 hr) at room temperature using proposed method and mean percentage recovery was determined.

Finally, validated UV method was applied for the estimation of total drug content in the marketed formulation of domperidone (Domperi DT 10 mg tablets) and in-house developed pellets containing 30 mg of domperidone.

3.3. Results and discussion

3.3.1. Optimization of media

The composition and pH of selected aqueous media decided was 100 mM hydrochloric acid buffer (pH 1.2) based on drug solubility, sensitivity of the method, cost, ease of

preparation and applicability of the method to dissolution studies. The λ_{max} of domperidone was found to be 284 nm with maximum absorbance.

3.3.2. Calibration curve

Six different concentrations were prepared in the range of 5-35 µg ml^{-1} of domperidone in the 100 mM HCl medium for calibration curve development. The obtained calibration data is presented in Table 3.1. The linear regression equation obtained was: absorbance = 0.0289 × concentration (µg ml^{-1}) + 0.0077; with regression coefficient of 0.9997. At all the concentration levels the SD was low and the %RSD did not exceed 1.30. Apparent molar absorptivity and Sandell's sensitivity of drug was found to be 1.27 × 10^4 l $mol^{-1}cm^{-1}$ and 0.034336 µg cm^{-2}/ 0.001A respectively which indicated good sensitivity of proposed method.

Table 3.1. Calibration curve data of proposed domperidone UV method

Drug concentration (µg ml^{-1})	**Absorbance at 284 nm ± SD[a] *(N = 9)***	**%RSD[b]**
5	0.1518 ± 0.002	1.295
10	0.2976 ± 0.001	0.238
15	0.4418 ± 0.002	0.477
20	0.5858 ± 0.003	0.541
25	0.7309 ± 0.009	1.199
35	1.0200 ± 0.009	0.829

[a] Standard deviation

[b] Relative standard deviation

3.3.3. Analytical method validation

The summary of various optical characteristics, statistical data of the regression equations and validation parameters is shown in Table 3.2. During selectivity investigation, no difference in absorbance was observed within UV absorption spectra recorded for drug solutions prepared with absence and the presence of selected excipients. In addition, UV absorption spectrum of pure drug sample was found to be matching with spectrum of marketed formulation (Figure 3.1). Further, the calculated t-values were found to be less than that of the critical t-value, which indicates that statistically there was no significant difference between the mean absorbance of domperidone solutions prepared from pure drug and marketed tablet formulation (Table 3.2). These results indicate that the proposed method is specific and selective for domperidone.

Table 3.2. Optical characteristics, statistical data for regression equations and validation parameters of domperidone UV method

Parameters	**Results**
Optical characteristics	
Apparent molar absorptivity (l mol^{-1} cm^{-1})	1.27×10^4
Sandell's Sensitivity (µgcm^{-2}/0.001A)	0.034336
Regression analysis	
Slope (S. E.[a])	0.02891 (2.35×10^{-5})
95% confidence limit of slope	0.02877 to 0.02906
Intercept (S. E.[a])	0.007806 (4.8×10^{-4})
95% confidence limit of intercept	0.004865 to 0.01075
Regression coefficient (R^2)	0.9997
Standard error of estimate	0.005098
One-way ANOVA for calibration curve	
Calculated F-value (Critical F-value)[b]	0.00047 (2.15)
Validation parameters	
Specificity and selectivity [t_{Cal} (t_{Crtic})][c]	0.4822 (2.132)
Linearity (µg ml^{-1})	5 to 35
Detection limit (µg ml^{-1})	0.3998
Quantification limit (µg ml^{-1})	1.2115
Robustness (mean % recovery ± SD)	99.76 ± 0.463

[a] Standard error of mean.

[b] Theoretical value of F(8,45) based on one-way ANOVA test at P = 0.05 level of significance.

[c] t_{Cal} is calculated value and t_{Crit} is a theoretical value (at 4 d.f.) based on paired t-test at P = 0.05 level of significance.

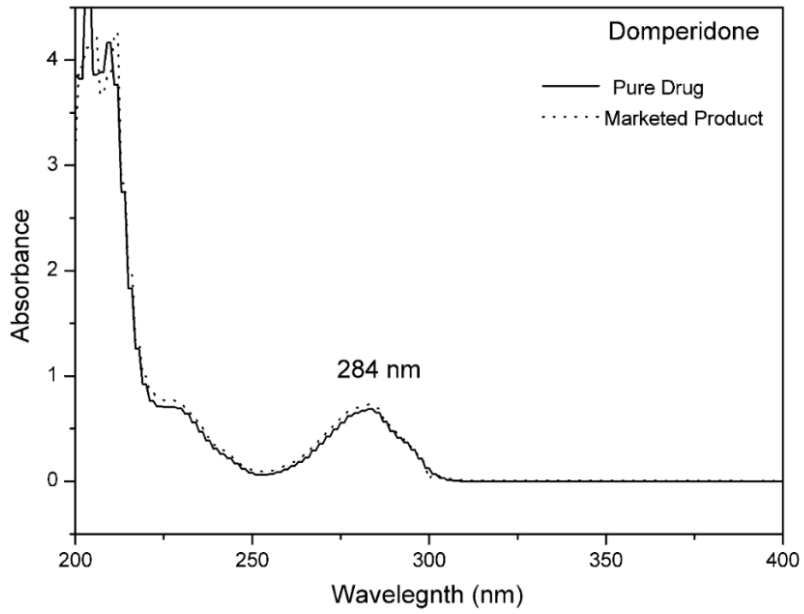

Figure 3.1. UV absorption spectra of domperidone in bulk form and marketed formulations (Solid line – pure drug; Dotted line - drug in marketed formulation)

The method was found to be linear over drug concentration range of 5-35 µg ml^{-1}. Lower values of parameters like standard error of slope and intercept (Table 3.2) indicated high accurcay and precision of the proposed method. Regression coefficient values close to 1 and less calculated F-values demonstrates the goodness of fit of regression equations.

The developed method showed high and consistent absolute recoveries at all studied QC levels. All the quality control levels (LQC = 7 µg ml^{-1}, MQC = 17 µg ml^{-1} and HQC = 27 µg ml^{-1}) showed an accuracy (% bias) ranging from -0.12 to 0.77 in 100 mM hydrochloric acid media. The mean recoveries (%RSD) for LQC, MQC and HQC were found to be 100.11 (1.10), 100.09 (0.79) and 100.05 (0.89) respectively (Table 3.3). Further, the accuracy of proposed method was validated by recovery values near 100 % with low %RSD values in standard addition method (Table 3.4). These result shows that the proposed method can accurately measure any small change in the drug concentration in solution.

Table 3.3. Results of accuracy studies by percentage recovery of domperidone UV method (each value is result of nine separate determinations)

Level	Predicted concentration (µg ml^{-1})			Mean % recovery ± SD	% Bias
	Range	Mean ± SD	%RSD		
LQC	6.93 - 7.07	6.99 ± 0.057	0.821	100.11 ± 1.103	-0.165
MQC	16.97 - 17.24	17.13 ± 0.083	0.482	100.09 ± 0.791	0.769
HQC	26.72 - 27.10	26.97 ± 0.150	0.557	100.05 ± 0.889	-0.114

Table 3.4. Results of accuracy studies by standard addition method for domperidone UV method (each value is result of three separate determinations)

Drug in formulation (µg ml^{-1})	Pure drug added (µg ml^{-1})	Actual total concentration of drug (µg ml^{-1})	Mean predicted concentrations (µg ml^{-1})	Mean % recovery ± SD
10	5	15	14.96	99.72 ± 0.832
10	10	20	20.01	100.05 ± 0.754
10	15	25	25.04	100.16 ± 0.576

The precision of the proposed method was studied by evaluating inter-instrument repeatability and intermediate precision. The %RSD of inter-day and intra-day precision was found to be less than 1.40 at all three QC levels of concentrations as shown in Table 3.5. Low %RSD values indicated the excellent precision of the proposed method.

The LOD and LOQ were found to be 0.40 µg ml^{-1} and 1.21 µg ml^{-1} respectively which indicate the excellent sensitivity in UV spectroscopic method.

Variation of pH (± 0.2) did not have any significant effect on the UV absorbance of domperidone, which confirms the robustness of the proposed method (Table 3.2).

Table 3.5. Results of precision studies of domperidone UV method

Concentration (µg ml^{-1})	Intra-day repeatability %RSD (*N* = 9)			Inter-day repeatability %RSD (*N* = 27)	Inter-instrument repeatability %RSD (*N* = 6)
	Day 1	Day 2	Day3		
LQC	0.791	1.176	1.396	0.921	0.661
MQC	0.475	0.565	0.463	0.572	0.588
HQC	0.552	0.474	0.270	0.454	0.567

Stability of the drug was investigated in 100 mM hydrochloric acid (pH 1.2). Results of stability study is compiled in Table 3.6. Mean percentage recoveries after 72 hr were found within limits which confirmed that domperidone is stable in selected solvent media for sufficient time.

The assay values of domperidone for different formulations ranged from 99.01 to 101.09% with SD not more than 1.10 (Table 3.7). Assay values of formulations were found to be very close to the labelled claim, suggesting that the interference of excipient matrix was insignificant in the estimation of domperidone by proposed methods. Low SD values indicate the precision and applicability of the proposed method.

Table 3.6. Stability study data for QC samples after 72 hr in selected media for domperidone UV method (each value is result of five separate determination)

Concentration (µg ml^{-1})	Predicted concentration ± SD	Mean % recovery ± SD
LQC	6.94 ± 0.072	99.18 ± 1.029
MQC	17.12 ± 0.105	100.75 ± 0.622
HQC	26.95 ± 0.111	99.83 ± 0.412

Table 3.7. Application of the proposed UV method for the estimation of domperidone in dosage forms (each value is result of six separate determinations)

Formulations	Amount found ± SD	% Assay ± SD
Marketed formulation: Domperi DT 10 mg tablets	10.02 ± 0.05	100.23 ± 0.561
In-house pellets containing 30 mg of domperidone	29.95 ± 0.33	99.84 ± 1.104

B. RP-HPLC method for the estimation of domperidone

3.4. Experimental

3.4.1. Materials and reagents

Domperidone (assay ≥ 99.78%) was purchased from Lee Pharma Ltd., India. A marketed formulation containing 10 mg of domperidone (Domperi DT, IPCA Laboratories Ltd., India) was purchased from the local Indian market. Orthophosphoric acid (assay ≥ 85%) and potassium dihydrogen orthophosphate (assay > 99.5%) were purchased from Merck Ltd., India. HPLC grade acetonitrile (assay > 99.7%) was purchased from Merck Ltd., India and Rankem Pvt. Ltd., India. All other chemicals and reagents used were of analytical grade and purchased from SD Fine-Chem Ltd., India. Ultrapure water was obtained using Millipore purification assembly (Mill-Q Plus, Millipore Corporation, USA).

3.4.2. Instrument details

The liquid chromatography system used was Shimadzu LC-2010HT (Shimadzu Corp., Tokyo, Japan) with a pulse-free solvent delivery system comprising of two pumps utilizing micro-volume serial double plunger design, high-efficiency 5-line degasser, block heating-type column oven, sample cooler (LC-2010CHT), intelligent autosampler and dual wavelength UV-visible detector. Separations were carried out on Phenomenex Hpyerclone 5µ BDS C18 (250 × 4.6 mm) reversed-phase analytical column. Data collection and integration were accomplished using LCsolution software.

3.4.3. Analytical method optimization

Various preliminary trials were taken to optimize the mobile phase composition, achieve desirable peak properties (peak area, retention time, tailing factor and height to area ratio), ease of sample preparation and the best applicability of the method for intended purpose. Varied composition of mobile phases with different proportions of organic solvent (methanol and acetonitrile) and aqueous phases (10 mM-25mM phosphate buffer at pH 3-6 and 10 mM citrate buffer at pH 3-5) were evaluated. After the optimization, the optimal mobile phase was further assessed for the effect of different flow rate (0.8-1.5 ml min^{-1}) as well as injection volumes (10-100µl) and adjusted for the best sensitivity and peak symmetry.

3.4.4. Preparation of mobile phase

The phosphate buffer (25 mM, pH 3.0) was prepared by dissolving 3.4 g of potassium dihydrogen phosphate in 1000 ml of ultrapure water. The pH of the solution was adjusted to 3.0 with the help of equimolar orthophosphoric acid. The mobile phase was prepared by taking 720 ml of the prepared buffer and 280 ml of acetonitrile. Before use, the mobile phase was filtered through 0.22 μm membrane filter and sonicated for 20 min.

3.4.5. Preparation of stock and standard solutions

Primary stock of domperidone was prepared by dissolving 10 mg in phosphate buffer (25 mM, pH 3.0) and making volume up to 100 ml to obtain a concentration of 100 μg ml^{-1}. Primary stock of domperidone was further diluted to give concentration of 10 μg ml^{-1}. This was further diluted to give concentration of 1 μg ml^{-1}. Working standard solutions in the range of 10 to 750 ng ml^{-1} (10, 25, 50, 100, 250, 500 and 750 ng ml^{-1}) were prepared by spiking appropriate volumes of the stock solution of domperidone to 2 ml centrifuge tube containing suitable volume of phosphate buffer (25 mM, pH 3.0).

3.4.6. Method validation

The developed method was validated in terms of system suitability, limit of detection, limit of quantification, specificity, linearity and range, precision, accuracy, stock solution stability and robustness as per USP and ICH guidelines [6-7].

3.4.6.1. System suitability

Five replicates of the standard concentration of 500 ng ml^{-1} were injected into the LC system to check the suitability of HPLC system for the intended application. The parameters measured were retention time, capacity factor, number of theoretical plates, tailing factor and peak area.

3.4.6.2. Sensitivity

The sensitivity of the proposed method was determined by using standard deviation of intercept (σ) and slope (s) obtained from the equation of the calibration curve. Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3.3 σ/s and 10 σ/s respectively.

3.4.6.3. Specificity

Specificity is the ability of an analytical method to measure the analyte, free from interference due to excipients, impurities and other matrices. To evaluate the specificity and selectivity of the proposed LC method, placebo and spiked-placebo analysis

techniques were used. On three consecutive days, placebo pellets and in-house developed pellet formulation were used for preparation of samples using same sample preparation process as described in the preparation of calibration standards. Prepared samples were then analyzed using the proposed method. The obtained chromatograms were checked for peak area. Further, interference of excipients and impurities were also examined at the retention time of the drug peaks.

In a separate study, drug concentration of 500 ng ml^{-1} was prepared for pure drug and marketed formulation in phosphate buffer (25 mM, pH 3.0) and analyzed (N = 5). Paired t-test at 95% level of significance was used to compare the means of absorbance.

3.4.6.4. Accuracy

Accuracy of the method was determined by performing the placebo spiking method and standard addition method. In the placebo spiking method, a known amount of standard solution of domperidone was added to placebo blank at five concentrations level (10, 50, 100, 500 and 750 ng ml^{-1}). In the standard addition technique, a known amount of pure drug was added to the sample solution at 50, 100 and 150% concentration level of the labeled claim of previously analyzed commercial capsule formulation of domperidone. All concentration levels were prepared in six replicates and accuracy was assessed as the mean percentage recovery and percentage bias {% Bias = 100 [(Predicted concentration – Nominal concentration)/Nominal concentration]}.

3.4.6.5. Precision

The intra-day and inter-day precision studies were carried out by estimating the responses of three quality control (QC) standards, covering the specified range, in triplicates under same experimental conditions three times on the same day and on three different days. Three quality control (QC) levels of drug concentration viz. lower quality control (LQC = 10 ng ml^{-1}), medium quality control (MQC = 150 ng ml^{-1}) and higher quality control (HQC = 700 ng ml^{-1}) were prepared independently from the stock solution. From the results obtained, the precision was expressed as percentage relative standard deviations (%RSD) from mean intraday and inter-day assays.

3.4.6.6. Robustness

Robustness is ability of analytical method to remain unaffected by small but deliberate change in optimized method parameters. This study was carried out using design of experiment (DOE) technique because it is an effective tool for obtaining maximum

information with minimum number of batches. In DOE, fractional factorial designs with resolution III and IV are generally used for screening, robustness and ruggedness studies. For this study, fractional factorial design with resolution III was selected as design model, as it allows identification of critical parameters with minimum number of trials. The effect of six method parameters viz. buffer pH, buffer strength, fraction of acetonitrile in mobile phase, flow rate, column temperature, and source of acetonitrile on chromatographic parameters was analyzed for robustness study of the proposed HPLC method. Key chromatographic parameters viz. peak area (mV), retention time (min) and tailing factor were selected as response. Design-Expert® (version 10.0.7, Stat-Ease Inc., Minneapolis, MN, USA) software was used for designing of experiment and all mathematical calculation.

3.4.6.7. Stability

To assess the stability of domperidone in the stock solution, QC samples were prepared in triplicate and stored at room temperature for 72 hr. Analysis was carried out at 0, 12, 24, 36, 48 and 72 hr. The % recovery and %RSD were calculated and reported.

3.4.6.8. Forced degradation study

For forced degradation studies, domperidone was subjected to hydrolytic stress (acid hydrolysis and base hydrolysis), oxidation, thermal and light degradation. The hydrolytic study was performed using 1N hydrochloric acid (acidic hydrolysis) and 1N sodium hydroxide (basic hydrolysis) at 65 ºC for 8 hr. Oxidation degradation study was carried out using 3% v/v hydrogen peroxide at 65 ºC for 8 hr. For thermal degradation, domperidone stock solution was exposed to 80 ºC temperature for 24 hr. Further, light degradation study was carried out by exposing domperidone stock solution to UV and fluorescent lamp for 10 days. All the solutions were diluted appropriately with phosphate buffer (25 mM, pH 3.0) and injected into the HPLC system for analysis. Hydrolytic study solutions were neutralized before dilution. All forced degradation studies were carried out in triplicate.

3.4.6.9. Application of method in drug content estimation

The developed and validated method was tested for the estimation of total drug content in the marketed formulation and in-house prepared pellets formulations. Mean absolute recoveries were calculated from five independent determination for both formulations.

3.5. Results and Discussion

3.5.1. Analytical method optimization

After investigation of various compositions of the mobile phase, a mixture of 25 mM phosphate buffer (pH 3.0) and acetonitrile in the proportion of 72:28 v/v respectively, provided the best chromatographic performance. Aliquots of extracted sample (40 µl) were injected into the system with a constant flow rate of 1 ml min^{-1} of mobile phase. The UV-detector was operated at a wavelength of 205 nm. The temperature of the column oven was maintained at 40 ºC. The run time of the proposed assay was 10 min under isocratic elution. A retention time of 6.8 min was observed with injections of standard solutions of domperidone (Figure 3.2)**.**

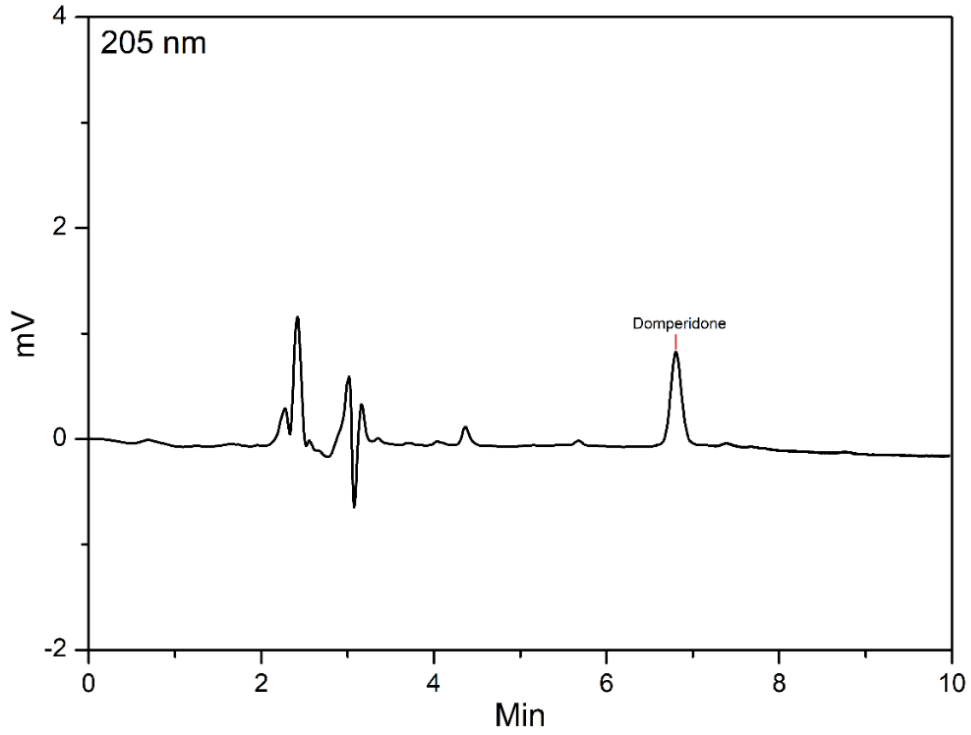

Figure 3.2. Representative chromatogram of domperidone at LOQ (6 ng ml^{-1})

3.5.2. Validation of the method

The validation study allowed the evaluation of the method for its suitability in routine analysis. The method was validated as per the USP and ICH guidelines. Forced degradation studies were also performed on domperidone to demonstrate the stability indicating power of the newly developed LC method.

3.5.2.1. System suitability

The results of system suitability showed excellent chromatographic peak parameters such as capacity factor (k > 2.0), number of theoretical plates (N > 12000) and tailing factor

(Tf ≤ 1.2). The typical chromatograms obtained from the final optimized HPLC conditions are depicted in Figure 3.2. The obtained peak parameters were well within the acceptable limits indicating the suitability of the method. Low variability in peak area and retention time were observed upon re-injection indicating that the developed method was highly suitable for estimation of domperidone.

3.5.2.2. Linearity

The results of regression analysis are as shown in Table 3.8. These results confirm the linearity of standard curves over the studied range (10-750 ng ml^{-1}). Linear regression of concentration versus peak area plots resulted in an average coefficient of determination (R^2) greater than 0.9999. The equation for calibration curve was y = 577x − 158.21. At all concentration levels of the calibration curve, standard deviation obtained was very low with %RSD less than 1.47%. The one-way ANOVA performed on peak area at each concentration level, indicated that the calculated F-value (6.23×10^{-6}) was far less than the critical F-value (2.12) at 5% level of significance.

Table 3.8. Calibration curve data of domperidone using proposed HPLC method (each value is result of nine separate determination)

Concentration (ng ml^{-1})	%RSD	Predicted concentration (ng ml^{-1})	% relative error
10	1.47	10.16	1.62
25	1.12	24.97	-0.11
50	1.46	50.02	0.04
100	0.83	100.64	0.64
250	0.52	251.27	0.51
500	0.46	495.35	-0.93
750	0.57	752.60	0.35

3.5.2.3. Sensitivity

The LOD and LOQ of the method were found to be 1.76 and 5.35 ng ml^{-1} respectively. Insignificant changes in chromatographic peak properties (retention time and peak area) were observed upon re-injection at quantification limit. Figure 3.2 shows the chromatogram at LOQ level which is well differentiated from baseline noise. These results prove that the method was highly sensitive for determination of domperidone.

Table 3.9. Statistical data summary for chromatographic method for domperidone

Parameters	**Results**
Calibration range (ng ml^{-1})	10 - 750
Linearity (Regression coefficient)	0.9999
Regression equation	y = 577 x + 158.21
Slope (S.E.[a])	577.2 (0.2949)
Confidence interval of slope[b]	575.6 to 578.7
Intercept (S.E.[a])	158.2 (105.07)
Confidence interval of intercept[b]	-397.6 to 714.1
Standard error of estimate	1.626×10^3
Specificity and selectivity - t_{Cal} (t_{Crit})[c]	0.3566 (2.015)
Limit of detection (ng ml^{-1})	1.77
Limit of quantification (ng ml^{-1})	5.35
Absolute recovery (Accuracy)	99.49 - 100.51%
Precision (%RSD[d])	Repeatability: 1.79% (Intraday) Intermediate precision: 1.90% (Interday)
System suitability	System precision: 0.48% (n = 10) Tailing factor: 1.12 Number of plates: 12539.457 HETP= 11.96 µm

[a] Standard error of mean

[b] Calculated at 0.05 level of significance

[c] t_{Cal} is calculated value and t_{Crit} is a theoretical value (at 5 d.f.) based on paired t-test at P=0.05 level of significance

[d] Relative standard deviation

3.5.2.4. Specificity

In specificity study, interference with the estimation of domperidone using the developed method was assessed by comparison of chromatograms solution of inactive ingredients (placebo solution) and standard drug solution (Figure 3.3). These chromatograms showed a single peak for domperidone, which indicates that there was no interference from the excipients used and also from the mobile phase. In a separate study, means of peak areas of pure drug and marketed formulation were compared by paired t-test at 95% confidence interval. The calculated t-values (t_{Cal} = 0.3556) was found to be lesser than the critical t-value (t_{Crit} = 2.015), indicated that there was no statistically significant difference between the mean peak areas of pure drug sample and marketed formulation samples (Table 3.9).

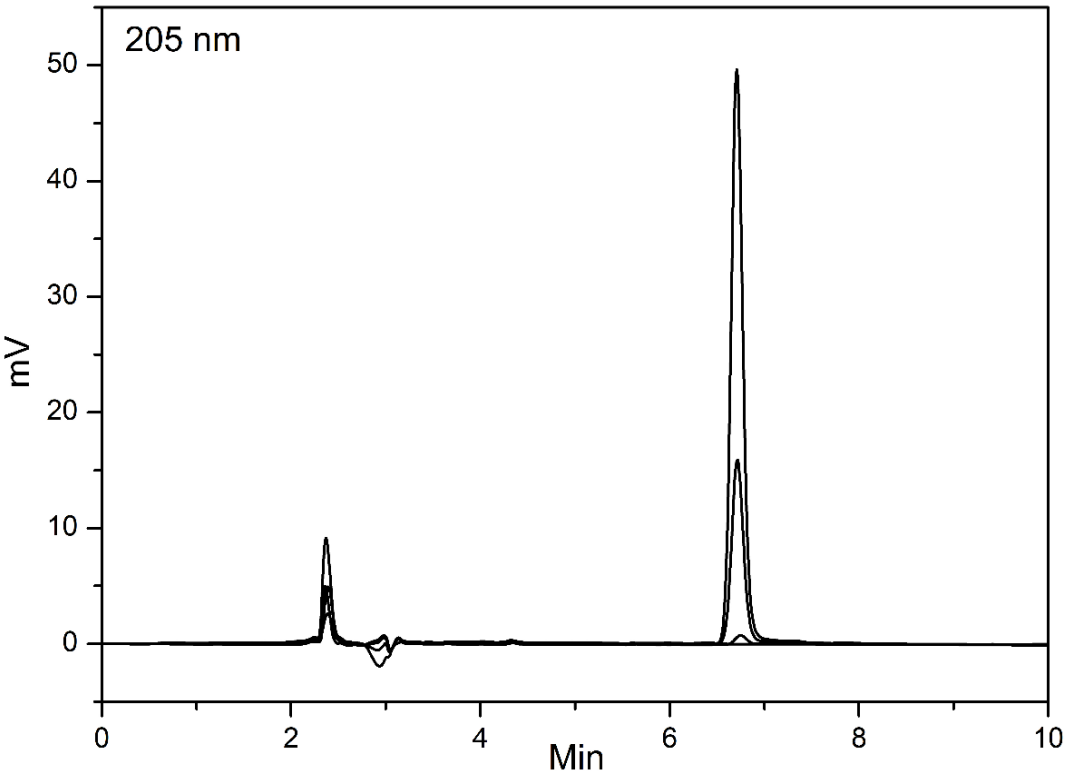

Figure 3.3. Chromatogram for placebo overlaid with QC standards for domperidone (LQC, MQC and HQC)

3.5.2.5. Accuracy

The developed method showed high and consistent absolute recoveries at all studied levels. The accuracy results obtained from standard addition method and placebo spiking method are presented in Table 3.10 and 3.11. The mean absolute recovery ranged from 99.49 to 100.51% with lower %RSD (≤1.67) at all concentration levels. These results prove that method is accurate, precise and can detect small changes in concentration with any interferences.

Table 3.10. Accuracy study by placebo spiking for domperidone HPLC method (each value is result of six separate determinations)

Amount of drug added (ng ml^{-1})	Mean amount recovered (ng ml^{-1})	Mean absolute recovery (%)	%RSD	Bias (%)
10	10.15 ± 0.12	100.51	1.44	0.51
50	49.89 ± 0.61	100.05	1.67	0.05
100	100.53 ± 0.57	100.18	0.91	0.18
500	502.60 ± 3.03	100.52	0.60	0.52
750	749.99 ± 3.61	100.23	0.54	0.23

Table 3.11. Accuracy study by standard addition for domperidone HPLC method (each value is result of six separate determinations)

Drug in formulation (ng ml^{-1})	Pure drug added (ng ml^{-1})	Actual total concentration of drug (ng ml^{-1})	Mean predicted concentrations (ng ml^{-1}) ± SD	Mean % recovery ± SD
200	100	300	299.32 ± 3.50	99.77 ± 1.17
200	200	400	400.99 ± 3.79	100.25 ± 0.95
200	300	500	497.45 ± 5.80	99.49 ± 1.16

3.5.2.6. Precision

Results of precision study is summarized in Table 3.12. In the intra-day and inter-day precision study, variation in the measured response of three quality control levels was found to be insignificant with %RSD values below 1.91%. These low %RSD values indicate that propose HPLC method is highly precise.

Table 3.12. Results of precision study for domperidone HPLC method

Conc. (ng ml^{-1})	Mean predicted concentration ± SD; %RSD			
	Intra-day precision (N = 9)			Interday precision (N = 27)
	Day 1	Day 2	Day 3	
10	10.17 ± 0.18; 1.78	10.08 ± 0.18; 1.80	10.12 ± 0.19; 1.91	10.13 ± 0.16; 1.62
150	150.69 ± 0.86; 0.57	150.37 ± 1.32; 0.88	150.82 ± 1.41; 0.94	150.62 ± 1.11; 0.73
700	702.85 ± 3.78; 0.54	700.68 ± 3.92; 0.56	701.13 ± 5.99; 0.85	701.55 ± 4.25; 0.61

3.5.2.7. Robustness

For robustness study, a fractional factorial design (resolution III) with six independent factors at two level was performed. Selected method parameters i.e. independent factors and their levels is summarized in Table 3.13. Experimental runs generated for this design and their observed response are summarized in Table 3.14A and 3.14B respectively. Half normal plots and pareto charts obtained for the effect of selected parameters on peak properties is shown in Figure 3.4. These are the primary tools for the selection of parameters which have significant effect on dependent parameters. In Figure 3.4A, Shapiro-Wilk p-value was found to be greater than 0.10 for peak area (p-value: 0.888)

without selecting any parameter which indicates that no parameter has significant effect on peak area.

Table 3.13. Fractional factorial design for robustness study of domperidone HPLC method: selected method parameters and their level

Sr. No.	Selected method parameters	Levels	
		Low (-1)	High (1)
1	pH	2.9	3.1
2	Buffer strength (mM)	23	27
3	Fraction of acetonitrile (%)	27	29
4	Flow rate (ml min^{-1})	0.9	1.1
5	Column temperature (ºC)	38	42
6	Source of acetonitrile	Merck	Rankem

Table 3.14A. Fractional factorial design for robustness study of domperidone HPLC method: experimental runs

Run	pH	Buffer strength (mM)	ACN (%)	Flow rate (ml min^{-1})	Column Temperature (ºC)	Source of acetonitrile
1	2.9	27	27	0.9	42	Merck
2	3.1	27	29	1.1	42	Rankem
3	2.9	23	29	1.1	38	Merck
4	3.1	23	27	0.9	38	Rankem
5	3.1	27	27	1.1	38	Merck
6	3.1	23	29	0.9	42	Merck
7	2.9	27	29	0.9	38	Rankem
8	2.9	23	27	1.1	42	Rankem

Table 3.14B. Fractional factorial design for robustness study of domperidone HPLC method: observed response

Run	Peak area (mv)	Retention time (min)	Tailing factor
1	281200	7.74	1.1185
2	286544	6.12	1.1151
3	287389	5.95	1.3331
4	284627	7.54	1.1354
5	283513	6.72	1.0912
6	284441	6.56	1.1235
7	283640	6.45	1.1223
8	286517	6.59	1.1313

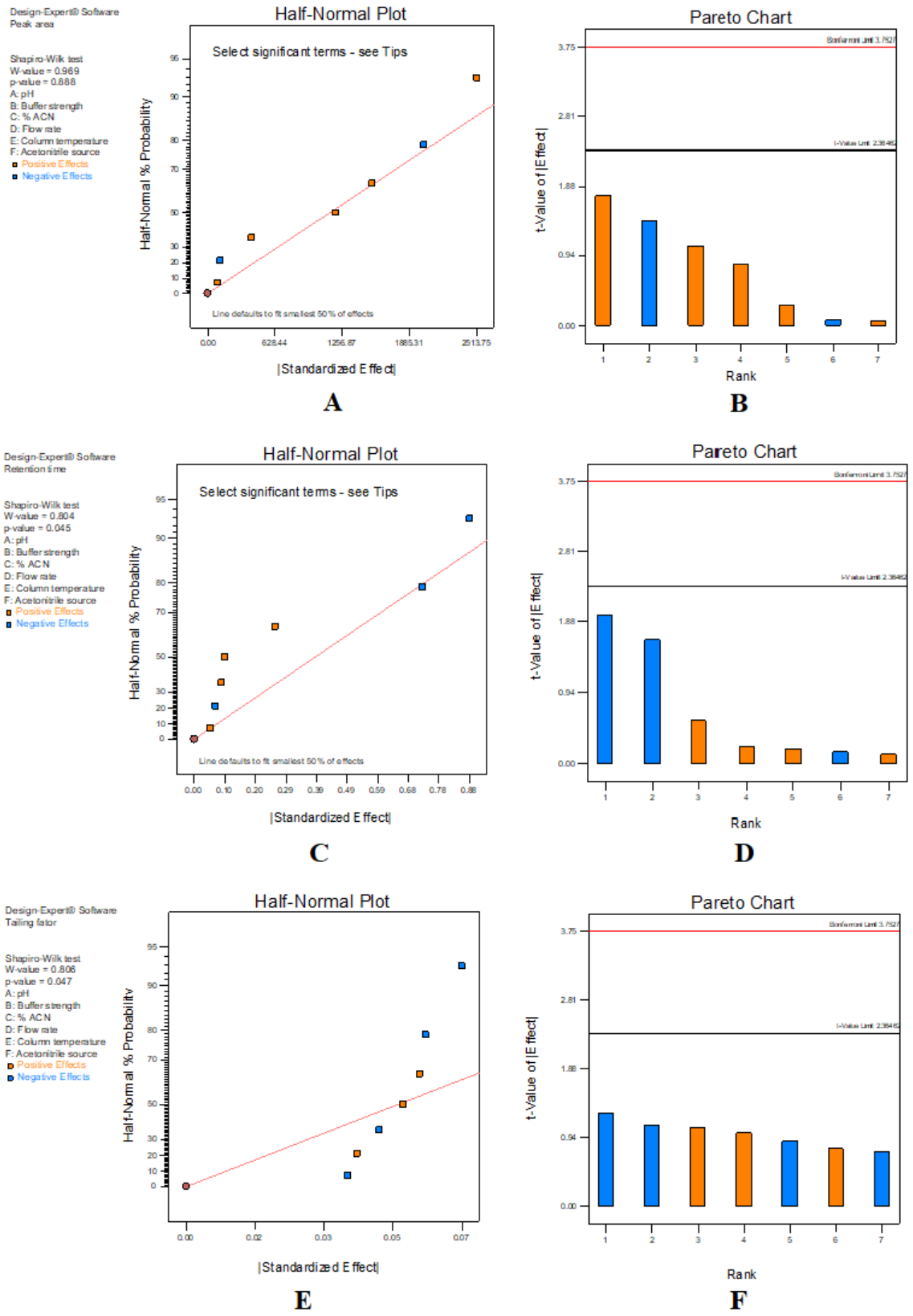

Figure 3.4. Fractional factorial design for robustness study of domperidone HPLC method: half normal plot for effect of method parameters on A. Peak area, C. Retention time, E. Tailing factor; Pareto chart for effect of method parameters on B. Peak area, D. Retention time, F. Tailing factor

In Figure 3.4C and 3.4E, Shapiro-Wilk p-value of less than 0.10 suggest that some of method parameters may have significant effect on retention time and tailing factor. However, in pareto charts obtained for all three chromatogram properties, effect of selected method parameters was found to be below t-value limit which suggest that none of the selected method parameters has significant effect on chromatogram of domperidone. Further, ANOVA study on linear models suggested that no selected parameter has significant effect on chromatographic properties. These results suggest that estimation of domperidone is not affected by small changes in any method parameters which confirms the robustness of proposed HPLC method.

3.5.2.8. Forced degradation study

Forced degradation studies were also carried out to evaluate the specificity of the developed method to distinguish the drug from its degradation products. The method is able to separate the drug from its degradation products as seen from the hydrolytic, oxidative, thermal and photo-degradation studies. The results obtained from forced degradation studies are summarized in Table 3.15. Degradation was observed in standard solutions exposed to hydrolytic stress (acidic and basic) stress however there was no additional peak observed for degradants (Figure 3.5). No degradation was observed under oxidation stress, thermal stress and photolytic stress. These results demonstrate that proposed method can be used for stability and compatibility studies.

Table 3.15. Forced degradation study for domperidone

Degradation method	Procedure	Observation	% Recovery ± SD
Acid hydrolysis	1N HCl/65 ºC/ 8 hr	Degradation overserved but no additional peak was observed	65.49 ± 0.87
Base hydrolysis	1N NaOH/65 ºC/ 8 hr	Degradation overserved but no additional peak was observed	91.42 ± 0.52
Oxidation	3% (v/v) H_2O_2/ 65 ºC/8 hr	No degradation observed	99.83 ± 1.13
Thermal	80 ºC/24 hr	No degradation observed	99.73 ± 1.47
Light	10 days in UV + fluorescent lamp	No degradation observed	100.36 ± 0.24

3.5.2.9. Stock solution stability

Stability data for stock solutions of domperidone in mobile phase after 96 hr is shown table 3.16. The recovery value for all QC level demonstrate the stability of the drug in

mobile phase. Low standard deviation values for predicted concentrations and recovery values further validate the robustness and precision of proposed HPLC method**.**

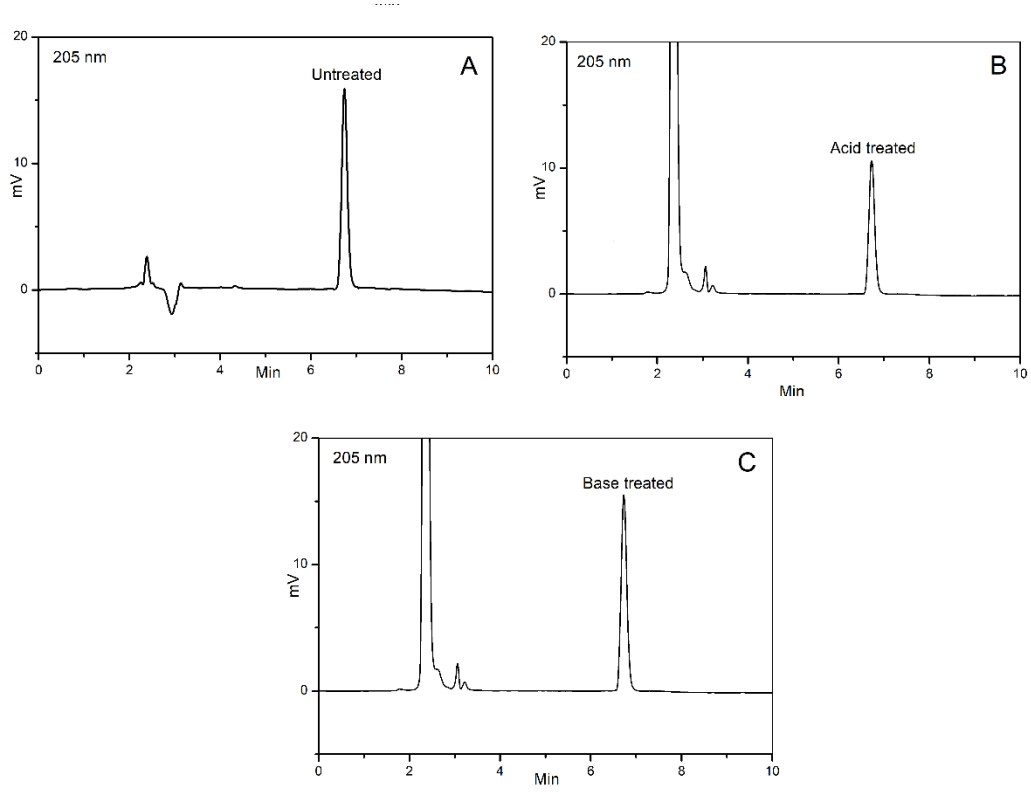

Figure 3.5. Chromatogram for forced degradation study A) Untreated sample B) Acid treated sample C) Base treated sample

Table 3.16. Stability studies of QC samples after 96 hr of domperidone using proposed HPLC method (each value is result of six separate determination)

Concentration (µg ml^{-1})	Predicted concentration ± SD	Mean % recovery ± SD	Bias (%)
10	10.05 ± 0.16	100.53 ± 1.21	0.53
150	150.42 ± 1.08	100.28 ± 0.72	0.28
700	703.66 ± 1.85	100.52 ± 0.26	0.52

3.5.2.10. Method application

The proposed validated LC method was successfully applied for the estimation of domperidone in marketed formulation Domperi DT 10 mg tablets. The in-house developed controlled release matrix pellets formulations were also analyzed using the developed method. The assay results are as summarized in Table 3.17. The good recovery 99.04 to 100.28% and low %RSD (0.42) values indicated that the assay results were satisfactory, accurate and precise. So, proposed method is specific and can be used for the estimation of domperidone in formulations without any interferences.

Table 3.17. Application of the proposed HPLC methods to the determination of domperidone in dosage forms (each value is result of six separate determinations)

Formulations	Amount found ± SD; %RSD	% Assay ± SD; %RSD
Marketed formulation: Domperi DT 10 mg tablets	9.94 ± 0.03; 0.34	99.39 ± 0.34; 0.34
In-house pellets containing 30 mg of domperidone	29.91 ± 0.33; 0.42	99.71 ± 0.42; 0.42

C. Bioanalytical method for the estimation domperidone in rat plasma

3.6. Experimental protocol

3.6.1. Reagents and chemicals

Domperidone (assay ≥ 99.78%) was purchased from Lee Pharma Ltd., India. Rivastigmine (assay ≥ 99%) was obtained as a gift sample from B. V. Patel PERD Center, India. A marketed formulation containing 10 mg of domperidone (Domperi DT, IPCA Laboratories Ltd., India) was purchased from the local Indian market. HPLC grade acetonitrile (assay > 99.7%), HPLC grade methanol (assay > 99.7%), potassium dihydrogen orthophosphate (assay > 99.5%) and orthophosphoric acid (assay ≥ 85%) were purchased from Merck Ltd., Mumbai, India. All other chemicals and reagents used were of analytical grade and purchased from SD Fine-Chem Ltd., India. HPLC grade water was produced using Milli-Q assembly (Mill-Q Plus, Millipore, MA, USA). Further, it was filtered further through a 0.22 µm Millipore membrane filter with the help of Millipore vacuum filtration system (Millipore, MA, USA) before using in HPLC system.

3.6.2. Instrumentation

The HPLC system (Shimadzu Corporation, Japan) consisted of a pulse-free solvent delivery system comprising two pumps (LC10AT VP), block heating-type column oven (CTO-10 AS), sample cooler (LC-2010CHT), intelligent autosampler (SIL HTA) and photodiode array (PDA) detector (SPD-M20A). Chromatographic peaks were collected and analyzed using LCsolutions software loaded on a computer system.

3.6.3. Chromatographic conditions

Chromatographic separations were carried out on Hpyerclone^{TM} 5µm BDS C18 LC column (250 mm long and 4.6 mm internal diameter, Phenomenex, USA). The optimized mobile phase consists of a mixture of phosphate buffer (10 mM, pH 3.0) and acetonitrile

in the ratio of 75:25 v/v. Aliquots of 50 µl were injected into the system with the mobile phase at a constant flow rate of 1.0 ml min^{-1}. The temperature of the column oven was maintained at 40 ºC. The detector was operated at a wavelength of 205 nm. The run time of the proposed LC method was 15 min under isocratic elution. Analysis was carried out after performing baseline stabilization for about 60 min.

3.6.4. Preparation of mobile phase

The phosphate buffer (10 mM, pH 3.0) was prepared by dissolving 3.4 g of potassium dihydrogen phosphate in 1000 ml of ultrapure water. The pH of the solution was adjusted to 3.0 with the help of equimolar orthophosphoric acid. The mobile phase was prepared by taking 750 ml of the prepared buffer and 250 ml of acetonitrile. Before use, the mobile phase was filtered through 0.22 µm membrane filter and sonicated for 20 min.

3.6.5. Blood samples collection and plasma separation

For bioanalytical method development, rats were anaesthetized in diethyl ether chamber and about 0.5 ml of blood was withdrawn from retro-orbital plexus. The blood was collected in 1.5 ml centrifuge tube containing 10% w/v ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) solution and centrifuged at 10,000 rpm for 15 min at 4 ºC temperature. The clear supernatant plasma was collected and stored at -80 ºC till its analysis. Rats were used (for blood sample collection) with the approval of Institutional Animal Ethics Committee (IAEC) of Birla Institute of Technology and Sciences, Pilani, Rajasthan, India (Protocol approval numbers IAEC/RES/18/12, IAEC/RES/18/12/Rev-1/19/31).

3.6.6. Plasma sample processing - solid phase extraction technique

Cleanert® PEP solid phase extraction cartridges (Bonna-Agela Technologies Inc., USA) were used for sample preparation. The cartridge was preconditioned twice with 1 ml methanol at 2000 rpm for 1 min. Then cartridge equilibration was carried out using 1 ml of phosphate buffer (10 mM, pH 3.0) at 2000 rpm for 1 min. The plasma sample was loaded and centrifuged at 2000 rpm for 3 min. After that cartridge was washed with 1 ml of 1% v/v methanol solution in water at 2000 rpm for 1 min. Finally, analytes were eluted with 2 ml of methanol at 2000 rpm for 1 min. Methanol was evaporated under nitrogen and analytes were reconstituted in the mobile phase. 150 µl aliquot of the reconstituted analytes solution was injected into the HPLC column. Blank plasma samples, plasma samples spiked with analytes were processed in a similar manner.

3.6.7. Preparation of standard solutions and calibration standards in plasma

Accurately weighed 10 mg of domperidone was dissolved in phosphate buffer (10 mM, pH 3.0) to obtain primary stock solutions of 100 µg ml^{-1} concentration. This was further diluted to get various working standard solutions of domperidone (1, 2, 20, 40, 60 and 80 µg ml^{-1}). Similarly, a primary stock solution of internal standard (IS), rivastigmine, was prepared in water and working standard of 12 µg ml^{-1} was made from it. Six calibration standards of domperidone in plasma (25, 50, 500, 1000, 1500 and 2000 ng ml^{-1}) were prepared by mixing 5 µl of working standard solutions of domperidone and 5 µl of working standard solutions of IS in 190 µl blank rat plasma. QC samples in plasma were prepared separately in plasma in a similar way as that of calibration standards in plasma prepared. All plasma samples were processed using solid phase extraction technique as described earlier.

3.6.8. Analytical method validation

Analytical method developed in rat plasma was validated for specificity, linearity, accuracy, precision, recovery, sensitivity, and drug stability in plasma. Validation was carried out as per the USFDA and European medicine agency (EMA) guidelines [8-9].

3.6.8.1. Specificity, selectivity and sensitivity

Selectivity of the method can be defined as absence any interference of the proteins or other impurities present in the biological matrix at the retention time of the analytes. The specificity and selectivity of the developed method was assessed by analyzing blank plasma obtained from six different rats. Chromatograms obtained from blank rat plasma samples were compared with analytical and calibration standards for investigating the interference in the determination of analyte. According to USFDA guideline [8] sensitivity is defined as the lowest analyte concentration that can be measured with acceptable accuracy and precision (RSD < 20%) which is also considered as lower limit of quantitation (LLOQ). The LLOQ sample of domperidone in plasma was prepared five times and analyzed by proposed method.

3.6.8.2. Calibration curve, linearity and range

Linearity of the method was determined by processing and analyzing different calibration standards (25 to 2000 ng ml^{-1}) prepared in rat plasma using working standard solutions of domperidone. Each concentration was prepared and analyzed in duplicate for three different days. A graph was plotted between ratio of drug and IS peak area and respective

concentrations of domperidone and data was subjected to linear least square regression analysis.

3.6.8.3. Recovery

The extraction efficiency of method was assessed as the recovery of domperidone from spiked rat plasma samples. It was determined by comparing ratio of drug and IS peak areas obtained from processing and analyzing QC samples (LLOQ, LQC, MQC and HQC) with those of the reference analytical samples. This experiment was performed six times to establish the recovery and it was expressed as % area ratio of extracted domperidone relative to ratio of drug and IS peak areas of reference standard.

3.6.8.4. Accuracy and precision

Accuracy and precision was determined by analyzing six replicates of four levels QC samples (LLOQ, LQC, MQC and HQC) for three consecutive days. Concentration of domperidone was estimated using freshly generated calibration curve. The inter-batch and intra-batch precision was reported as %RSD while accuracy was reported as % recovery and % bias.

3.6.8.5. Stability

All stability studies of domperidone in rat plasma were carried out by analyzing six replicates of QC samples at lower (60 ng ml^{-1}) and higher (1800 ng ml^{-1}) concentration levels. The stability was determined at different levels such as freeze and thaw stability, bench top stability, long term stability, and post preparative stability in the autosampler. In freeze and thaw stability study, three cycles of freezing and thawing (-80 ºC to room temperature) were performed before determining the stability. For bench top stability studies, QC samples were kept at room temperature and each set was processed and analyzed at 0, 6, 12 and 24 hr. Likewise, long term stability of domperidone in plasma was assessed at -80 ºC for a period of 90 days, with analysis time points of 7, 15, 30, 60 and 90 days. Finally, for post preparative stability in the autosampler, QC samples were processed and stored in the HPLC autosampler at 4 ºC for 48 hr with analysis time points of 0, 12, 24 and 48 hr. Concentration of domperidone in stability sample was determined using freshly generated calibration curve. The results were expressed as % recovery and % bias.

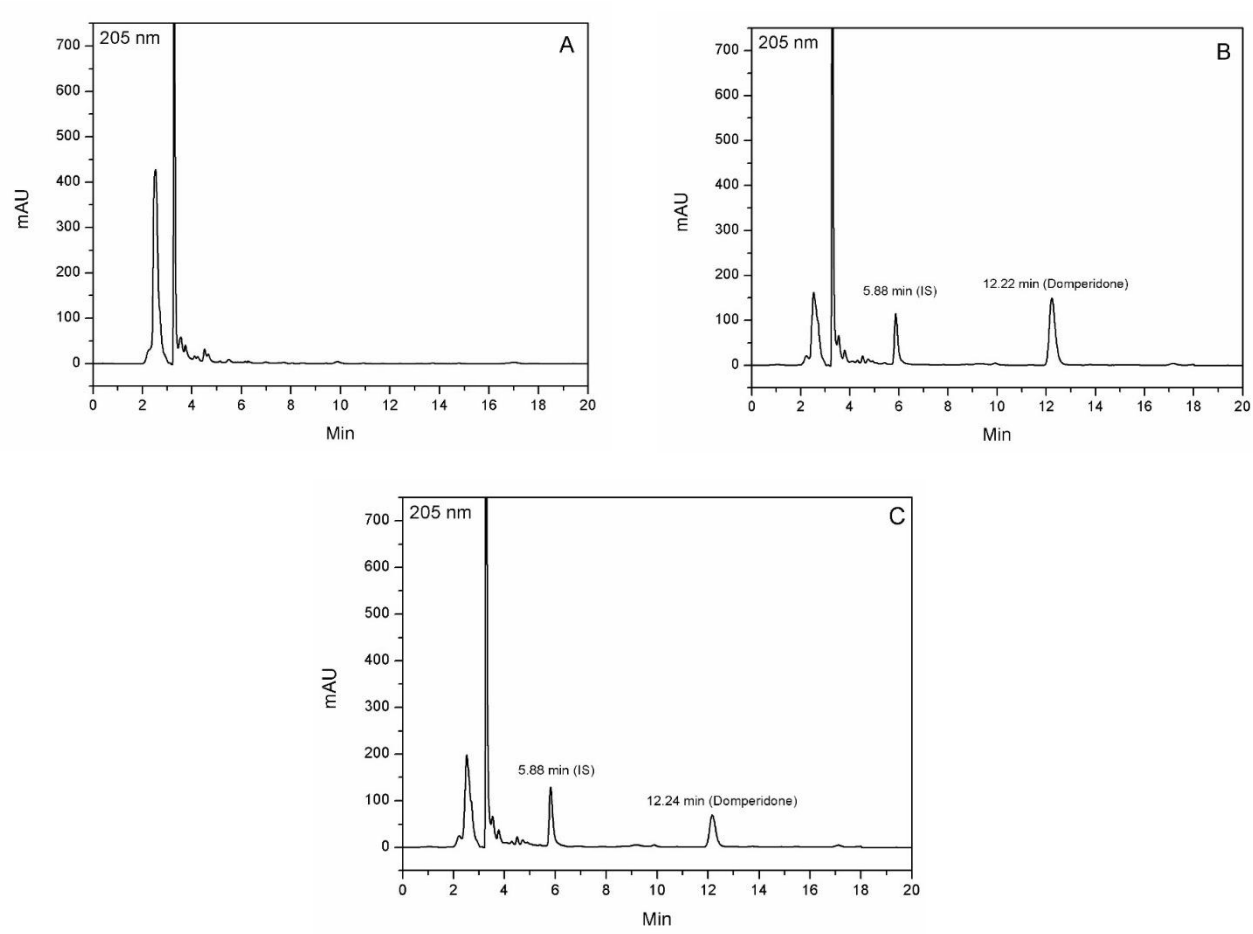

Figure 3.6. Representative chromatograms of (A) blank plasma, (B) spiked plasma sample, and (C) test sample (plasma sample from rats after 4 hr administered with sustained release pellets of domperidone)

3.7. Result and discussion

3.7.1. Analytical method optimization

For the optimization of mobile phase, various compositions of the mobile phase have been evaluated. A mixture of 10 mM phosphate buffer (pH 3.0) and acetonitrile in the ratio of 75:25 v/v respectively was finalized since it has provided the best chromatographic performance. Sample injection volume 50 µl with mobile phase flow rate of 1 ml min^{-1} was finalized as it provided acceptable sensitivity and peak properties (peak area, retention time, tailing factor and height to area ratio). The UV-detector was operated at a wavelength of 205 nm.

Preliminary trials for various extraction methods (i.e. protein precipitation technique with acetone, perchloric acid, trichloroacetic acid as well as solid phase extraction techniques) were carried out. Solid phase extraction method with Cleanert® PEP solid phase extraction cartridges was selected as it provided highest recovery. Rivastigmine was

selected as internal standard as it exhibited well resolved peak, similar extraction character, stability as well as acceptable sensitivity for selected method. The run time of the proposed assay was 15 min under isocratic elution.

3.7.2. Specificity, selectivity and sensitivity

The chromatograms obtained after processing and analyzing blank plasma sample, indicated that there was no interfering peak within the vicinity of the analyte peaks. A lack of response to blank biological matrix originating from endogenous components in the elution window of the analytes demonstrated the selectivity of the proposed method. Comparison of chromatograms of blank plasma samples (Figure 3.6A), plasma samples spiked with IS well as a drug (Figure 3.6B) and test samples obtained from rats after drug administration (Figure 3.6C) proved that there was no interference from the plasma proteins, metabolites or degradation products to the drug peaks. These results prove that the proposed method was found to be selective and specific for determination of domperidone in rat plasma samples. To determine the LLOQ, five independent rat plasma samples containing 25 ng ml^{-1} of domperidone were prepared, processed and analyzed by the proposed method. The observed % recovery value was found to be 79.82% with %RSD of lower than 7.24%. The lower values %RSD and acceptable absolute recovery demonstrate that the proposed method was a sensitive and can be used for pharmacokinetic study in rat plasma which requires higher sensitivity.

3.7.3. Calibration curve, linearity and range

Calibration data of proposed method for the estimation of domperidone in rat plasma is summarized in Table 3.18. The linearity of the proposed method was observed over the range of 25 to 2000 ng ml^{-1}. The best fit linear regression equation obtained was (at 205 nm for rat plasma sample): $y = 0.0011x + 0.0281$ with a regression coefficient of 0.9982.

Table 3.18. Calibration curve data of domperidone in rat plasma (each value is result of six separate determinations)

Drug concentration (ng ml^{-1})	Analyte/IS Ratio	%RSD
25	0.036961	12.51
50	0.075231	9.18
500	0.628568	4.56
1000	1.058791	3.83
1500	1.629721	3.38
2000	2.187526	3.50

3.7.4. Recovery

The extraction efficiency or recovery of the proposed method using solid phase extraction technique was found in the range of 79.82 to 84.97% at all QC levels. The %RSD was found to be not more than 7.24% (Table 3.19). These results prove that method has consistent and acceptable extraction efficiency for domperidone in rat plasma.

Table 3.19. Recovery study data of domperidone in rat plasma (each value is result of six separate determinations)

Drug concentration (ng ml^{-1})	Mean absolute recovery	%RSD
25	79.82	7.24
60	81.58	5.47
600	84.97	4.55
1800	82.58	2.45

3.7.5. Accuracy and precision

Accuracy studies data is represented in Table 3.20. Proposed method demonstrated high and consistent recoveries at all QC levels indicating the correctness of the method. Inter-batch and intra-batch precision studies data is summarized in Table 3.21. Low %RSD (≤7.56%) values at all QC levels demonstrate the reliability of the method.

Table 3.20. Results of accuracy studies of domperidone in rat plasma (each value is result of six separate determinations)

Level	Predicted concentration (µg ml^{-1})		Mean % recovery	Accuracy (%)
	Mean	%RSD		
25	24.68	8.82	98.72	-1.28
60	58.80	6.48	98.00	-2.00
600	589.50	5.55	98.25	-1.75
1800	1789.05	3.86	99.39	-0.61

Table 3.21. Precision study data for domperidone in rat plasma

Drug concentration (ng ml^{-1})	Intra-batch precision (%RSD) (N=6)	Inter-batch precision (%RSD) (N=18)
25	7.563	6.295
60	5.898	3.238
600	3.676	5.477
1800	4.734	3.541

3.7.6. Stability

Stability of domperidone in rat plasma was assessed under different storage and handling conditions which test samples may encounter during various processing steps from blood collection to analysis by HPLC. The results of these stability studies are summarized in Table 3.22. Domperidone in rat plasma samples were found to stable after 3 cycles of freeze thaw with % recovery in the range of -4.63 to -2.76%. The % recovery of bench top stability studies and long-term studies was found in the range of -3.55 to -1.72% and -6.40 to -4.28% respectively, indicating the stability of domperidone in rat plasma for 24 hr at room temperature and for 90 days at -80 ºC. In post preparative stability study, % recoveries were found the range of -5.85 to -2.10%, indicating the stability of plasma sample for 48 hr at 4 ºC in the autosampler of HPLC.

Table 3.22. Stability data of domperidone in rat plasma (Each result represents the average of six independent determinations)

Stability Type	QC levels	% recovery	% bias
Freeze thaw stability (3 cycles)	LQC	95.37	-4.63
	HQC	97.24	-2.76
Bench top stability (at room temperature for 8 hr)	LQC	96.45	-3.55
	HQC	98.18	-1.72
Long term stability (at -80 ºC for 90 days)	LQC	93.60	-6.40
	HQC	95.72	-4.28
Post-preparative stability studies (48 hr at 4 ºC in autosampler)	LQC	94.15	-5.85
	HQC	97.90	-2.10

Part II. Analytical methods for venlafaxine hydrochloride

A. UV spectroscopic method for venlafaxine hydrochloride

3.8. Experimental protocol

3.8.1. Reagents and chemicals

Venlafaxine hydrochloride (assay ≥ 99.83%) was purchased from Aarti Industries Ltd., India. A marketed formulation containing 75 mg of venlafaxine hydrochloride (Venla 75 mg capsule, Ranbaxy Laboratories Ltd., India) was purchased from the local Indian market. All other chemicals and reagents used were of analytical grade and purchased from SD Fine-Chem Ltd., India. Ultrapure water was obtained using Millipore purification assembly (Mill-Q Plus, Millipore, MA, USA).

3.8.2. Instruments

Spectroscopic measurements were performed using a double-beam UV-Vis-NIR spectrophotometer as described earlier in this chapter (Section 3.2.2.).

3.8.3. Analytical method development

In the preliminary study, ultrapure water, various aqueous buffers and organic solvents were screened to develop a suitable UV-spectrophotometric method for the analysis of venlafaxine hydrochloride. Media was selected based on the solubility of the drug, sensitivity, ease of sample preparation, economy of the method and its applicability. Drug solutions in the selected media were scanned in the range from 200-400 nm wavelengths for selecting the wavelength of analysis.

3.8.4. Calibration standards

200 μg ml^{-1} stock solution of venlafaxine hydrochloride was prepared in selected media by dissolving 20 mg of the drug in 100 ml of selected media. Different concentrations were prepared from stock solution by transferring aliquots of stock solutions into a series of 2 ml of the centrifuge tube and diluted suitably with selected media using micropipette. six different concentrations were prepared in the range of 5-35 μg ml^{-1} of venlafaxine hydrochloride in selected media for calibration curve development.

3.8.5. Analytical method validation

The developed method was validated in terms of specificity, selectivity, linearity, range, precision, accuracy, limit of detection, limit of quantification, and robustness as described earlier in this chapter (Section 3.2.5).

3.9. Results and discussion

3.9.1. Optimization of media

Ultrapure water was selected as media for method development based on drug solubility, sensitivity of method, cost, ease of preparation and applicability of method to dissolution studies. The λ_{max} of venlafaxine hydrochloride was found to be 224 nm with maximum absorbance.

3.9.2. Calibration curve

Six different concentrations were prepared in the range of 5-35 µg ml^{-1} of venlafaxine hydrochloride in ultrapure water for calibration curve development. The calibration data is shown in Table 3.23. The linear regression equation obtained was: absorbance = 0.0344 × concentration (µg ml^{-1}) + 0.0062 with a regression coefficient of 1. At all the concentration levels the SD was low and the % RSD did not exceed 1.14. Apparent molar absorptivity and Sandell's sensitivity of drug was found to be 9.60×10^3 l $mol^{-1}cm^{-1}$ and 0.02888 µg cm^{-2}/ 0.001A respectively which indicates the good sensitivity of the proposed method.

3.9.3. Analytical method validation

The summary of various optical characteristics, statistical data of the regression equations and validation parameters are shown in Table 3.24. In the selectivity investigation, the UV absorption spectrum of venlafaxine hydrochloride was not changed in the presence of common formulation excipients. In addition, UV absorption spectrum of pure drug sample was found to be matching with spectrum of marketed formulation (Figure 3.7)

Table 3.23. Calibration curve data of venlafaxine hydrochloride UV method (each value is result of nine separate determinations)

Drug concentration (µg ml^{-1})	Absorbance at 284 nm ± SD[a]	%RSD[b]
5	0.177 ± 0.002	1.145
10	0.350 ± 0.002	0.543
15	0.524 ± 0.003	0.633
20	0.696 ± 0.003	0.362
25	0.869 ± 0.002	0.195
35	1.210 ± 0.003	0.244

[a] Standard deviation
[b] Relative standard deviation

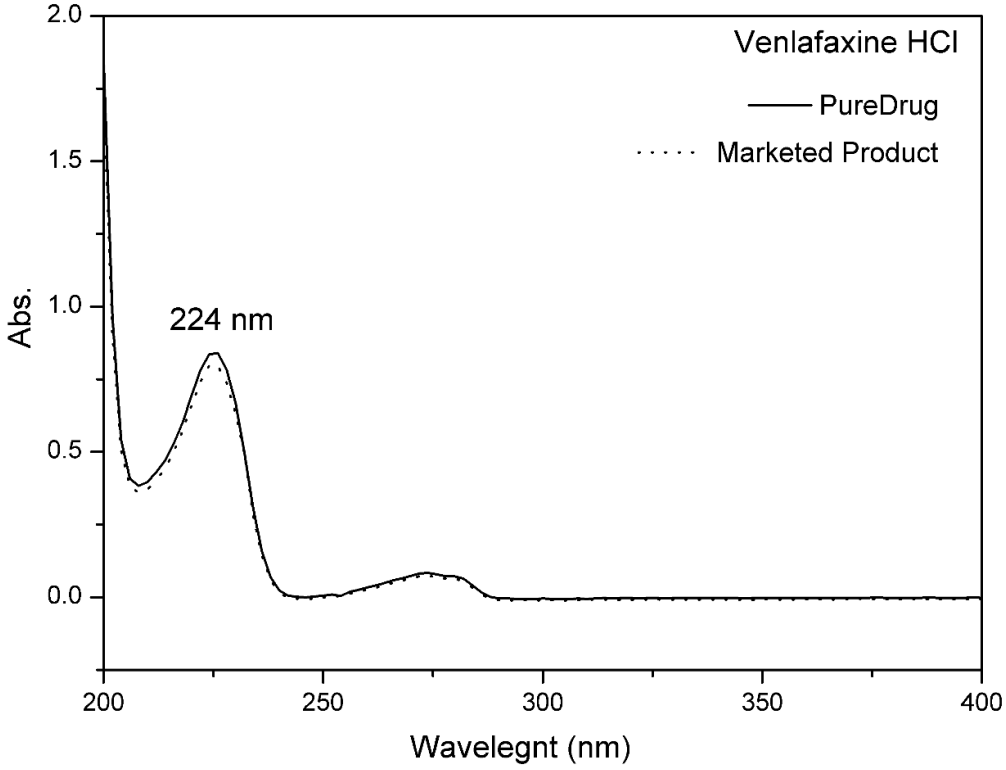

Figure 3.7. UV absorption spectra of venlafaxine hydrochloride in bulk form and marketed formulations (Solid line - pure drug; Dotted line - drug in marketed formulations)

Further, the calculated t-values were found to be less than that of the critical t-value, which indicates that statistically there was no significant difference between the mean absorbance of venlafaxine hydrochloride solutions prepared from pure drug and marketed tablet formulation (Table 3.24). Therefore, the proposed method is specific and selective for venlafaxine hydrochloride.

The linearity of proposed UV method was found to be in the drug concentration range of 5-35 µg ml^{-1}. Lower values of parameters like standard error of slope and intercept reveals that response data is scattered near to calibration curve which demonstrates high precision and high accuracy of the proposed UV method (Table 3.24). High regression coefficient values and less calculated F-value demonstrate the goodness of fit of regression equations.

The developed method showed high and consistent absolute recoveries at all studied QC levels. All the quality control levels (LQC = 9 µg ml^{-1}, MQC = 18 µg ml^{-1} and HQC = 27 µg ml^{-1}) showed an accuracy (% bias) ranging from 0.57 to 1.39% in selected solvent media (Table 3.25).

Table 3.24. Optical characteristics, statistical data of the regression equations and validation parameters of venlafaxine hydrochloride UV method

Parameters	**Results**
Optical characteristics	
Apparent molar absorptivity (l mol^{-1} cm^{-1})	9.60×10^3
Sandell's Sensitivity (µgcm^{-2}/0.001A)	0.02888
Regression analysis	
Slope (S. E.[a])	0.03444 (1.17×10^{-5})
95% confidence limit of slope	0.03437 to 0.03451
Intercept (S. E.[a])	0.006175 (2.62×10^{-4})
95% confidence limit of intercept	0.004598 to 0.007752
Regression coefficient (R^2)	1
Standard error of estimate	0.002799
One way ANOVA for calibration curve	
Calculated F-value (Critical F-value)[b]	0.00003 (2.15)
Validation parameters	
Specificity and selectivity [t_{Cal} (t_{Crtic})][c]	0.5898 (2.132)
Linearity (µg ml^{-1})	5 to 35
Detection limit (µg ml^{-1})	0.1023
Quantification limit (µg ml^{-1})	0.3102
Robustness (mean % recovery ± SD)	100.67 ± 0.1508

[a] Standard error of mean.

[b] Theoretical value of F (8,45) based on one-way ANOVA test at P = 0.05 level of significance.

[c] t_{Cal} is calculated value and t_{Crit} is a theoretical value (at 4 d.f.) based on paired t-test at P = 0.05 level of significance.

Further, the accuracy was validated by performance recovery studies of standard addition method (Table 3.26). The percentage analytical recoveries were found within range of 100.57 to 101.39% with %RSD less than 1.249%. These results indicate that the proposed method can accurately measure any minute changes in the drug concentration level in solution.

Table 3.25. Results of accuracy studies by percentage recovery for venlafaxine hydrochloride (each value is result of nine separate determinations)

Level	**Predicted concentration (µg ml^{-1})**			**Mean % recovery ± SD**	**Accuracy (%)**
	Range	**Mean ± SD**	**%RSD**		
LQC	8.98 - 9.24	9.13 ± 0.114	1.249	101.39 ± 1.266	1.39
MQC	17.93 - 18.40	18.14 ± 0.144	0.792	100.78 ± 0.798	0.78
HQC	26.72 - 27.10	27.16 ± 0.156	0.574	100.57 ± 0.577	0.57

Table 3.26. Results of accuracy studies by standard addition method for venlafaxine hydrochloride (each value is result of three separate determinations)

Drug in formulation (µg ml^{-1})	Pure drug added (µg ml^{-1})	Actual total concentration of drug (µg ml^{-1})	Mean predicted concentrations (µg ml^{-1})	Mean % recovery ± SD
10	5	15	14.97	99.77 ± 0.194
10	10	20	19.85	99.24 ± 0.145
10	15	25	24.88	99.94 ± 0.470

Precision of the proposed method was studied by evaluating repeatability and intermediate precision. The %RSD of inter-day and intra-day precision was found to be not more than 1.26% at all three QC levels of concentrations as shown in Table 3.27. Low %RSD values indicated the excellent precision of the proposed method.

The LOD and LOQ were found to be 0.1023 µg ml^{-1} and 0.3102 µg ml^{-1} respectively which indicate the excellent sensitivity in UV spectroscopic method. Variation of pH (± 0.2) did not have any significant effect on the UV absorbance of venlafaxine hydrochloride, which confirms the robustness of the proposed method (Table 3.24).

Table 3.27. Results of precision studies of venlafaxine hydrochloride UV method

Concentration (µg ml^{-1})	Intra-day repeatability %RSD (N = 9)			Inter-day repeatability %RSD (N = 27)	Inter-instrument repeatability %RSD (N = 6)
	Day 1	Day 2	Day3		
LQC	1.264	1.224	1.014	1.122	1.073
MQC	0.529	0.785	0.666	0.641	0.511
HQC	0.552	0.570	0.555	0.594	0.555

Further, stability of the venlafaxine hydrochloride was investigated in ultrapure water for 72 hr at room temperature. The results of stability study are summarized in Table 3.28. Mean percentage recoveries after 72 hr were found within limits which confirmed that venlafaxine hydrochloride is stable in selected solvent media for sufficient time.

Table 3.28. Stability study data for QC samples after 72 hr in selected media for venlafaxine hydrochloride UV method (each value is result of five separate determination)

Concentration (µg ml^{-1})	Predicted concentration ± SD	Mean % recovery ± SD
LQC	9.054 ± 0.073	100.60 ± 0.813
MQC	18.124 ± 0.044	100.69 ± 0.247
HQC	27.116 ± 0.201	100.43 ± 0.746

The assay values of venlafaxine hydrochloride for different formulations ranged from 99.83 to 100.26% with SD not more than 0.81 (Table 3.29). Assay values of formulations were found to be very close to the labeled claim, suggesting that the interference of excipient matrix was insignificant in the estimation of venlafaxine hydrochloride using proposed method. The estimated drug content with low values of SD establishes the precision and applicability of the proposed method.

Table 3.29. Application of the proposed spectrophotometric methods to the determination of venlafaxine hydrochloride in dosage forms (each value is result of six separate determinations)

Formulations	Amount found ± SD	% Assay ± SD
Marketed formulation: Venla (75 mg) capsule	74.96 ± 0.18	99.94 ± 0.81
In-house pellets containing 30 mg of venlafaxine hydrochloride	29.65 ± 0.24	98.83 ± 0.81

B. RP-HPLC method for venlafaxine hydrochloride

3.10. Experimental

3.10.1. Materials and reagents

Venlafaxine hydrochloride (assay ≥ 99.78%) was purchased from Aarti Industries Ltd., India. Marketed formulation containing 37.5 mg of venlafaxine hydrochloride (Venla-XR, Ranbaxy Laboratories Ltd., India) was purchased from the local Indian market. HPLC grade acetonitrile (assay > 99.7%), potassium dihydrogen orthophosphate (assay > 99.5%) and orthophosphoric acid (assay ≥ 85%) were purchased from Merck, Mumbai, India. All other chemicals and reagents used were of analytical grade and purchased from SD Fine-Chem Ltd., India. Ultrapure water was obtained using Millipore purification assembly (Mill-Q Plus, Millipore, MA, USA).

3.10.2. Chromatographic conditions

The HPLC system (LC-2010HT, Shimadzu Corporation, Japan) consisted of a pulse-free solvent delivery system comprising two pumps, high-efficiency 5-line degasser, block heating-type column oven, sample cooler (LC-2010CHT), intelligent autosampler and dual wavelength UV-visible detector. Chromatographic peaks were collected and analyzed using LCsolutions software loaded on a computer system. Chromatographic separations were carried out on Hpyerclone^{TM} 5µm BDS C18 LC column (250 mm long and 4.6 mm internal diameter, Phenomenex, USA). The optimized mobile phase consists

of a mixture of phosphate buffer (25 mM, pH 3.0) and acetonitrile in the ratio of 70:30 v/v. Aliquots of 50 µl were injected into the system with the mobile phase at a constant flow rate of 1.0 ml min^{-1}. The temperature of the column oven was maintained at 30 ºC. The detector was operated at a wavelength of 225 nm. The run time of the proposed LC method was 10 min under isocratic elution.

3.10.3. Analytical method optimization

Varied composition of mobile phases with different proportions of organic solvent (methanol and acetonitrile) and aqueous phases (10 mM-25mM phosphate buffer at pH 3-6 and 10 mM citrate buffer at pH 3-5) were evaluated. For optimization of the mobile phase, peak properties (peak area, retention time, tailing factor and height to area ratio), ease of sample preparation, applicability of the method for intended purpose were employed as the selection criteria. After selection of the optimal mobile phase, effect of different flow rate (0.8-1.5 ml min^{-1}) and injection volumes (10-100µl) were assessed and optimized for best sensitivity and peak symmetry.

3.10.4. Preparation of mobile phase

The phosphate buffer (25 mM, pH 3.0) was prepared by dissolving 3.4 g of potassium dihydrogen phosphate in 1000 ml of ultrapure water. The pH of the solution was adjusted to 3.0 with the help of equimolar orthophosphoric acid. The mobile phase was prepared by taking 700 ml of the prepared buffer and 300 ml of acetonitrile. Before use, the mobile phase was filtered through 0.22 µm membrane filter and sonicated for 20 min.

3.10.5. Calibration curve

Primary stock solution of 100 µg ml^{-1} of venlafaxine hydrochloride was prepared by dissolving 10 mg of drug in 100 ml of the phosphate buffer (25 mM, pH 3.0). A secondary stock of 100 µg ml^{-1} was prepared by taking an aliquot from the primary stock and diluting with the phosphate buffer (25 mM, pH 3.0). For developing the calibration curve, different calibration standards having concentrations of 100, 200, 400, 500, 1000, 1500 and 2000 ng ml^{-1} were prepared by taking appropriate aliquots of secondary stock solution in 1.5 ml centrifuge tubes and diluting them suitably with phosphate buffer (25 mM, pH 3.0). Calibration curve was plotted between peak area against the concentration of the venlafaxine hydrochloride. The data were subjected to least square regression analysis. ANOVA test (one-way) at 95% level of significance was performed based on

the peak area values observed for each pure drug concentration during the replicate measurement of the standard solutions.

3.10.6. Method validation

The developed method was validated for various parameters according to the standard guidelines [6-7]. The system suitability of the system for the intended application was checked, to ensure proper validity of the method, by injecting ten replicates of the standard preparation (500 ng ml^{-1}) into the LC system. The parameters measured were retention time, capacity factor, number of theoretical plates, tailing factor and peak area.

Specificity is the ability of an analytical method to measure the analyte, free from interference due to excipients, impurities and other matrices. To evaluate the specificity and selectivity of the proposed LC method, placebo and spiked-placebo analysis techniques were used. On three consecutive days, placebo pellets and in-house developed pellet formulation were used for the preparation of samples using same sample preparation process described in the preparation of calibration standards. Prepared samples were then analyzed using the proposed method. The obtained chromatograms were checked for peak area. Further, interference of excipients and impurities were also examined at the retention time of the drug peaks. In a separate study, drug concentration of 1000 ng ml^{-1} was prepared for pure drug and marketed formulation in phosphate buffer (25 mM, pH 3.0) and analyzed (N = 5). Paired t-test at 95% level of significance was used to compare the means of absorbance.

To determine the accuracy of the proposed method, placebo spiking method and standard addition method were used. In placebo spiking method, a known amount of drug was added to placebo blank to obtain the sample solutions with a concentration of 100, 200, 800, 1500 and 1800 ng ml^{-1}. In the standard addition method, marketed formulation sample solutions were prepared and analyzed. In that samples, a known amount of pure drug (50, 100 and 150% of total drug present in the marketed formulation sample) were added. All concentration samples were prepared in six replicates and accuracy was assessed as the mean percentage recovery and percentage bias {% Bias = 100 [(Predicted concentration – Nominal concentration)/Nominal concentration]}.

Three quality control (QC) levels of drug concentration viz. lower quality control (LQC = 200 ng ml^{-1}) samples, medium quality control (MQC = 800 ng ml^{-1}) samples and higher quality control (HQC = 1800 ng ml^{-1}) samples were prepared independently from

the stock solution. For precision study of the method, intra-day and inter-day precision studies were carried out by estimating the responses of these QC standards in triplicates under same experimental conditions three times on the same day and on three different days. From the results obtained, precision was expressed as percentage relative standard deviations (%RSD) from mean intra-day and inter-day assays.

The sensitivity of the proposed method was determined using the standard deviation of intercept (σ) and mean of slopes (s) obtained from the equation of calibration curve. Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3.3 σ/s and 10 σ/s respectively.

The chromatographic analysis was performed under different analytical conditions and the chromatographic parameters of the main peak were evaluated for studying the robustness of the method. Changes in acetonitrile concentration in the mobile phase (70 ± 5%), pH of buffer media (3 ± 0.5) and buffer strength (25 ± 5 mM) were evaluated for the robustness of proposed method.

The stability of venlafaxine hydrochloride in selected media was determined for 96 hr. Three QC standard (LQC, MQC and HQC) were prepared in triplicates and mean percentage recovery was obtained for each sample.

3.10.7. Forced degradation study

For forced degradation studies, venlafaxine hydrochloride was subjected to hydrolytic stress (acid hydrolysis and base hydrolysis), oxidation, thermal and light degradation. The hydrolytic study was performed using 1N hydrochloric acid (acidic hydrolysis) and 1N sodium hydroxide (basic hydrolysis) at 65 ºC for 8 hr. Oxidation degradation study was carried out using 3% v/v hydrogen peroxide at 65 ºC for 8 hr. For thermal degradation, venlafaxine hydrochloride stock solution was exposed to 80 ºC temperature for 12 hr. Further, light degradation study was carried out by exposing venlafaxine hydrochloride stock solution to UV and fluorescent lamp for 10 days hr. All the solutions were diluted appropriately with phosphate buffer (25 mM, pH 3.0) and injected into the HPLC system for analysis. Hydrolytic study solutions were neutralized before dilution. All forced degradation studies were carried out in triplicate.

3.10.8. Estimation of drug content in formulations

As a part of the validation protocol, the proposed LC method was tested for the estimation of total drug content in the marketed formulation (Venla-XR, Ranbaxy Laboratories Ltd.,

India) and in-house prepared pellet formulations. Mean absolute recoveries were calculated from five independent determination for both formulations.

3.11. Results and discussion

3.11.1. Analytical method optimization

In preliminary studies, we optimized the type of organic phase using methanol and acetonitrile with different proportions of the aqueous phase to achieve desired peak properties and response function. Peak properties were also affected by organic to aqueous phase ratio, buffer type, buffer strength and the pH of aqueous phase. After investigation of various compositions of the mobile phase, a mixture of 70% phosphate buffer (25 mM, pH 3.0) and 30% of acetonitrile as mobile phase provided the best chromatographic performance. To achieve good sensitivity with optimal peak symmetry, aliquots of 50 µl were injected into the system with optimized mobile phase at a constant flow rate of 1.0 ml min^{-1} and column oven temperature was maintained at 30 ºC. A retention time of 5.5 min was observed with injections of standard solutions of venlafaxine hydrochloride (Figure 3.8).

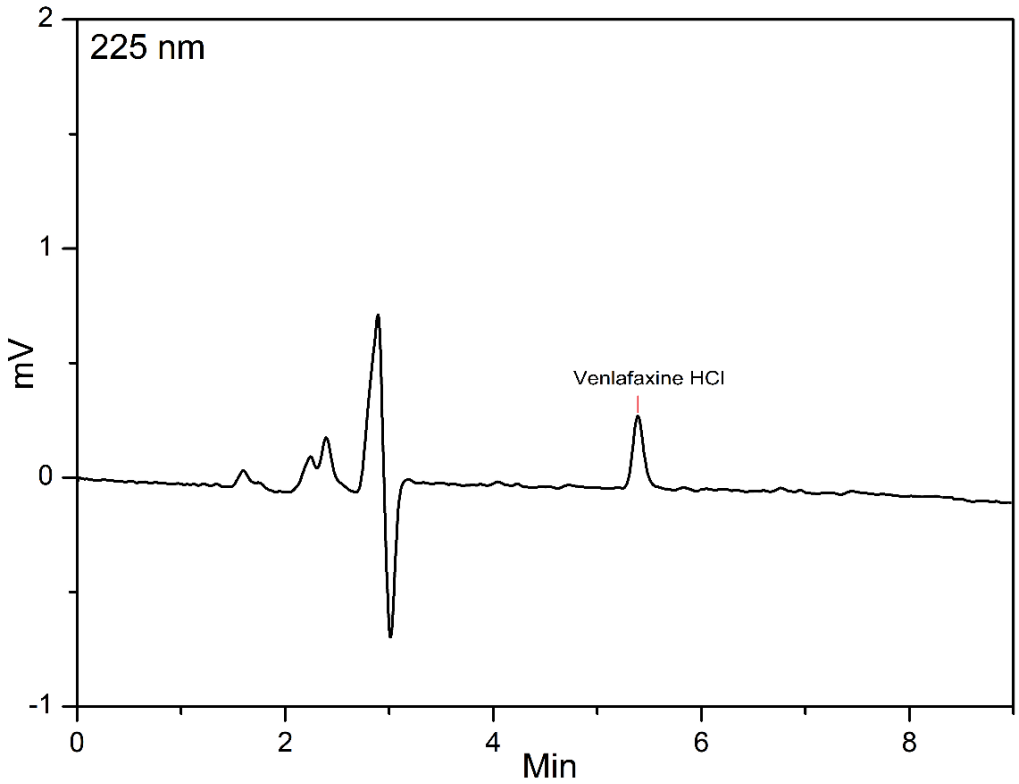

Figure 3.8. Representative chromatogram of venlafaxine hydrochloride at LOQ (20 ng ml^{-1}).

3.11.2. Calibration curve

Various drug concentrations and respective peak areas are reported in Table 3.30. The method was found to be linear over the range of 100 to 2000 ng ml^{-1}. According to linear regression analysis (Table 3.31), regression coefficient (R^2) was found to be 1. The average equation for calibration curves was $y = 93.321x - 322$. At all concentration levels of the calibration curve, standard deviation obtained was very low with %RSD less than 1.83%. One-way ANOVA performed on peak area at each concentration level, indicated that the calculated F-value (4.8×10^{-5}) was far less than the critical F-value (2.12) at 95% level of significance which further confirmed the precision of the proposed method.

Table 3.30. Calibration curve data of venlafaxine hydrochloride using proposed HPLC method (each value is result of nine separate determination)

Concentration (ng ml^{-1})	%RSD	Predicted concentration (ng ml^{-1})	% relative error
100	1.37	101.98	1.98
200	1.40	200.00	0.00
400	1.67	403.08	0.77
500	1.83	493.44	-1.31
1000	0.90	1000.05	0.00
1500	0.60	1502.22	0.15
2000	0.39	1999.23	-0.04

3.11.3. Method validation

The results of system suitability studies (Table 3.31) indicated that the method has shown excellent chromatographic peak parameters such as capacity factor ($k > 2.0$), number of theoretical plates (112111.27 to 11308.01) and tailing factor ($Tf \leq 1.12$). The obtained peak parameters were well within the acceptable limits indicating the suitability of the method. Low variability in peak area and retention time were observed upon re-injection indicating that the developed method was highly suitable for estimation of venlafaxine hydrochloride.

In specificity study, interference with the estimation of venlafaxine hydrochloride using the developed method was evaluated with a solution of inactive ingredients (placebo solution). The chromatogram showed the absence of peaks due to the inactive ingredients when a standard solution of venlafaxine hydrochloride and placebo were injected (Figure 3.9).

Table 3.31. Statistical data summary of chromatographic method for venlafaxine hydrochloride

Parameters	**Results**
Calibration range (ng ml^{-1})	100 to 2000
Linearity (Regression coefficient)	1
Regression equation	$y = 99.321x - 322$
Slope (S.E.[a])	93.32 (0.1325)
Confidence interval of slope[b]	93.06 to 93.59
Intercept (S.E.[a])	-322.0 (46.36)
Confidence interval of intercept[b]	-600.2 to -43.82
Standard error of estimate	6.966×10^2
Specificity and selectivity - t_{Cal} (t_{Crit})[c]	0.2963 (2.015)
Limit of detection (ng ml^{-1})	6.55
Limit of quantification (ng ml^{-1})	19.84
Absolute recovery (Accuracy)	99.18 to 101.16%
Precision (%RSD[d])	Repeatability: 1.79% (Intraday) Intermediate precision: 1.65% (Interday)
System suitability	System precision: 0.64% (n = 10) Tailing factor: 1.12 Number of plates: 112111.27 to 11308.01 HETP = 13.11 to 13.39 µm

[a] Standard error of mean

[b] Calculated at 0.05 level of significance

[c] t_{Cal} is calculated and t_{Crit} is a theoretical value (at 5 d.f.) based on paired t-test at P=0.05 level of significance

[d] Relative standard deviation

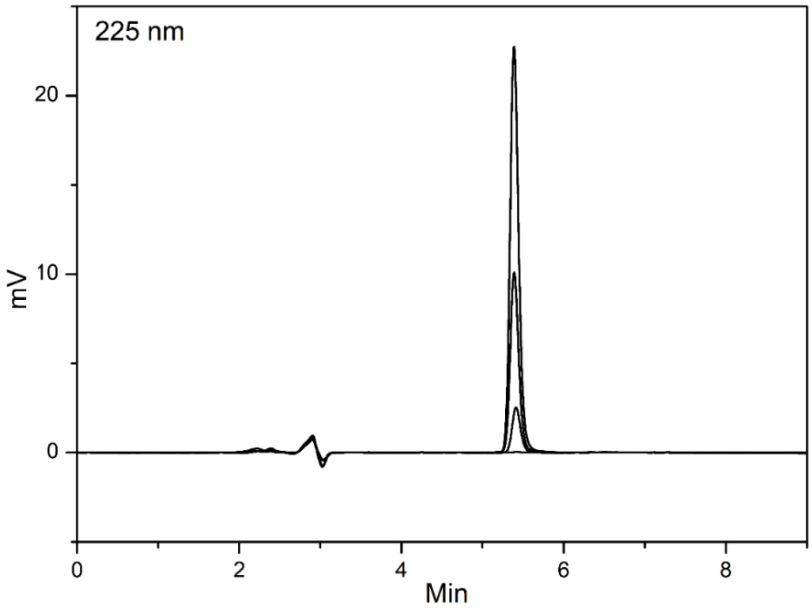

Figure 3.9. Chromatogram for placebo overlaid with QC standards for venlafaxine hydrochloride (LQC, MQC and HQC)

A single peak was obtained for venlafaxine hydrochloride, which indicates that there was no interference from the excipients used and from the mobile phase. The means of peak areas were compared by paired t-test at 95% confidence interval. The calculated t-values (t_{Cal} = 0.2963) was found to be lesser than the critical t-value (t_{Crit} = 2.015), revealing that there was no statistically significant difference between the mean peak areas of standards prepared from pure drug sample and pellets formulation sample (Table 3.31).

In accuracy studies, the developed method showed high and consistent absolute recoveries at all studied levels. The accuracy results obtained from standard addition method and placebo spiking method are presented in Table 3.32 and 3.33. The mean absolute recovery ranged from 99.18 to 101.16%. Additionally, the obtained recoveries were found to be precise with significantly low %RSD (≤ 1.55%) at all concentration levels.

Table 3.32. Results of accuracy study using placebo spiking method for venlafaxine hydrochloride HPLC method (each value is result of six separate determination).

Concentration of drug (ng ml^{-1})	Mean absolute recovery (%)	%RSD	% Bias
100	100.64 ± 1.09	1.08	0.64
200	100.80 ± 1.56	1.55	0.80
800	100.84 ± 0.83	0.83	0.84
1500	99.82 ± 0.99	1.00	-0.18
1800	99.34 ± 0.32	0.32	-0.66

Table 3.33. Results of accuracy study using standard addition method for venlafaxine hydrochloride HPLC method (each value is result of three separate determination).

Concentration of drug in formulation (ng ml^{-1})	Concentration of pure drug added (ng ml^{-1})	Mean amount recovered (ng ml^{-1}) ± SD	Mean absolute recovery (%) ± SD	% Bias
400	200	606.94 ± 7.73	101.16 ± 0.78	1.16
400	400	805.89 ± 8.00	100.74 ± 1.00	0.74
400	600	991.80 ± 9.62	99.18 ± 0.96	-0.82

The results of inter-day and intraday precision studies indicated high reproducibility with the %RSD values not more than 1.79%. The data obtained from precision studies is shown in Table 3.34. At all the standard concentration levels, variation observed was insignificant indicating the repeatability of the method.

Table 3.34. Precision study for venlafaxine hydrochloride HPLC method

Conc. (ng ml^{-1})	Mean predicted concentration ± SD; %RSD			
	Repeatability (intraday, N = 9)			Repeatability (interday, N = 27)
	Day 1	Day 2	Day 3	
200	200.66 ± 3.46; 1.73	202.27 ± 3.07; 1.52	201.59 ± 3.60; 1.79	201.50 ± 3.32; 1.65
800	806.94 ± 6.50; 0.81	803.67 ± 7.60; 0.95	806.14 ± 6.97; 0.86	805.59 ± 6.91; 0.86
1800	1815.45 ± 13.69; 0.75	1814.84 ± 15.76; 0.87	1816.25 ± 13.66; 0.75	1815.52 ± 13.85; 0.76

For sensitivity investigation, the LOD and LOQ of the method were found to be 6.55 and 19.85 ng ml^{-1} respectively. Hence the method was found to be highly sensitive for determination of venlafaxine hydrochloride.

The effects of variations in % of the organic solvent in the mobile phase, pH and buffer strength on the developed analytical method were evaluated in the robustness study. The percentage mean recovery was found in the range of 100.04 to 100.53% with %RSD lower than 1.38% under all the varied conditions. Thus, there was no significant effect on peak area and other peak properties indicating the robust nature of the developed method. The results of stability of venlafaxine hydrochloride in the selected media indicated that all the QC samples were found to be stable up to 96 hr with percentage mean recovery of 98.98 to 101.99% (Table 3.35).

Table 3.35. Stability studies of QC samples after 96 hr for venlafaxine hydrochloride HPLC method (each value is result of three separate determination).

Concentration (ng ml^{-1})	Predicted concentration ± SD	Mean % recovery ± SD	% Bias
200	198.98 ± 3.15	99.49 ± 1.57	-0.51
800	806.38 ± 7.69	100.80 ± 0.96	0.80
1800	1793.00 ± 14.98	99.61 ± 0.83	-0.39

3.11.4. Forced degradation study

Forced degradation studies were also carried out to evaluate the specificity of the developed method in distinguishing the drug from its degradation products. The results obtained from forced degradation studies are summarized in Table 3.36. Under acidic hydrolysis, the peak of venlafaxine hydrochloride showed mean percentage recovery of 85.26 ± 1.45% indicating the possibility of degradation but no interference or additional peak was observed (Figure 3.10). There was no significant degradation observed in other

stress conditions. Force degradation studies demonstrated that venlafaxine hydrochloride is more sensitive to strong acidic condition than other stress conditions. All these results indicate that method can separate the drug from the degradation products which proves stability indicating power of the proposed method.

Table 3.36. Summary of forced degradation studies for venlafaxine hydrochloride HPLC method

Degradation method	Procedure	Observation	% Recovery ± SD
Acid hydrolysis	1N hydrochloric acid/ 65 ºC/8 hr	Degradation observed	85.26 ± 1.45
Base hydrolysis	1N sodium hydroxide/ 65 ºC/8 hr	No degradation observed	99.42 ± 0.72
Oxidation	3% hydrogen peroxide/ 65 ºC/8 hr	No degradation observed	99.53 ± 1.21
Thermal	80 ºC/12 hr	No degradation observed	100.13 ± 0.98
Light	72 hr in UV and fluorescent lamp	No degradation observed	99.86 ± 0.84

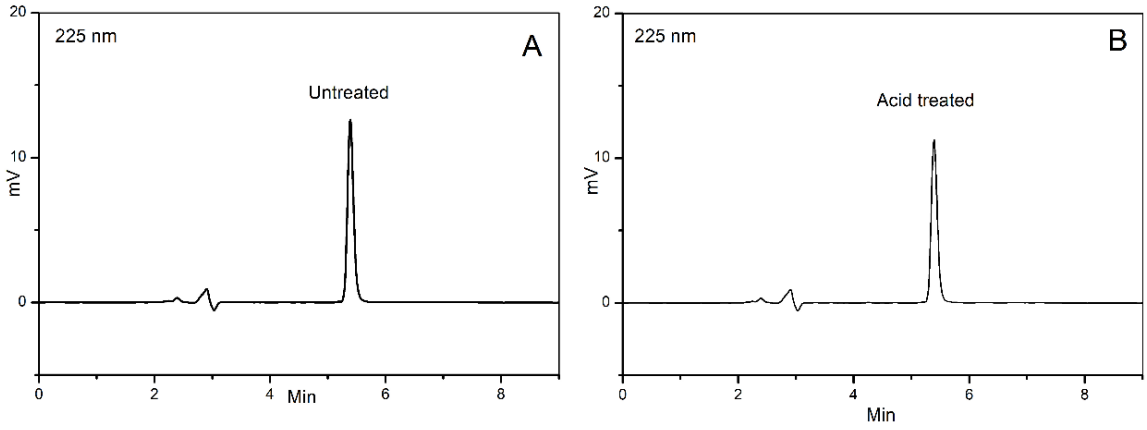

Figure 3.10. Chromatogram for forced degradation study for venlafaxine hydrochloride HPLC method A) Untreated sample B) Acid treated sample

3.11.5. Estimation of drug content in formulations

The proposed LC method was successfully employed for the estimation of venlafaxine hydrochloride in marketed tablet formulation and in-house developed pellet formulation. The results are as summarized in Table 3.37. A typical chromatogram for the estimation of venlafaxine hydrochloride extracted from a marketed tablet formulation is shown in Figure 3.11. Percentage recoveries for the estimation of venlafaxine hydrochloride in formulation were obtained in the range of 98.93 to 100.55% with %RSD not more than 0.54%. Good recovery values indicate accuracy of method and absence of interference

with drug estimation using proposed HPLC method. Low RSD values proves that method is precise and can be used for the analysis of formulation.

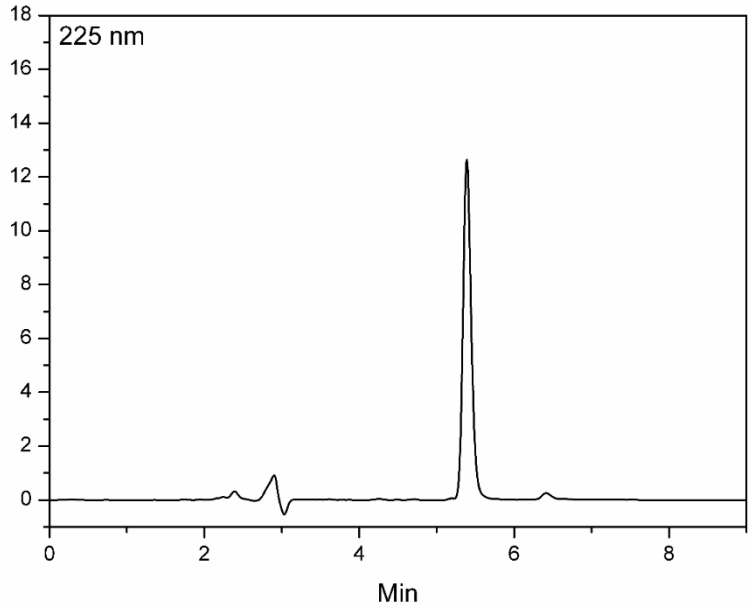

Figure 3.11. Chromatogram of marketed tablet formulation of venlafaxine hydrochloride

Table 3.37. Estimation venlafaxine hydrochloride from pharmaceutical formulations (each value is result of five separate determinations).

Formulation	Labelled amount of drug	Amount of drug recovered ± SD	% Recovery ± SD	%RSD
Marketed formulation Venla-XR	37.50 mg	37.45 ± 0.20	99.86 ± 0.54	0.54
In-house developed pellet formulation	37.50 mg	37.54 ± 0.17	100.11 ± 0.47	0.47

3.12. Conclusion

The proposed UV spectrophotometric methods are simple, rapid, accurate, precise and economical for the estimation of domperidone and venlafaxine hydrochloride in bulk, dosage form and dissolution samples. The UV methods have a wider linear range with good accuracy and precision. Thus, these UV spectroscopic methods can be extended for routine analysis of domperidone and venlafaxine hydrochloride in pharmaceutical industries and research laboratories.

The developed reverse phase HPLC methods for the estimation of domperidone and venlafaxine hydrochloride in bulk, formulations and analytical samples were found to be specific, simple and highly sensitive. Both HPLC methods were validated as per the ICH

guideline. Further, these HPLC methods were successfully employed for estimation of drug content of marketed and in-house formulations. In view of the above facts, these methods can be conveniently used for the routine quality control analysis of drug in both bulk as well as in its formulations. Furthermore, no interference was observed with the drug peak in the forced degradation study which demonstrates stability indicating power of the methods. Thus, the proposed HPLC methods can also be used for stability and drug-excipient compatibility studies.

In addition, a bioanalytical method using HPLC with UV detector was developed and validated for the estimation of domperidone in rat plasma. Solid phase extraction technique was used for processing of plasma samples which demonstrated excellent recoveries for domperidone. Validation studies proved that the proposed bioanalytical method was highly sensitive, selective, precise and accurate for the estimation of domperidone in rat plasma and can be used for in-vivo pharmacokinetic studies of domperidone formulations in a rat model.

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4. Preformulation Studies

4.1. Introduction

A successful formulation design considers the prior information of physiochemical and biological properties of the drug to develop safe, optimally bioavailable and stable formulation [1-3]. Preformulation studies have a significant role in anticipating potential barriers and identifying logical paths in formulation development process which minimizes efforts in the later stage of product design and development. Thus, it helps to reduce the cost of the product and time to reach the market. Although drug regulatory authorities have not released any specific guidelines for pellets, all investigations were carried out as per the standard product development guidelines. Usually, a standard pharmaceutical product development study includes comprehensive drug characterization such as determination of dissociation constant, partition coefficient, solubility, polymorphism and hydrates, powder properties, thermal behavior, molecular spectroscopic profile, drug-excipient compatibility studies, stability studies, etc. [4-6]. Domperidone and venlafaxine hydrochloride are well-established and widely prescribed drugs and thus, a lot of information about physico-chemical characteristics is available in the literature [7-12]. As our aim was to formulate modified release pellets using specific excipients, a few selected preformulation studies were still required for successful formulation design. Therefore, various preformulation experiments such as bulk characterization, pH solubility studies, drug-excipient compatibility and solution and solid stability studies were carried out.

4.2. Experimental

4.2.1. Materials

Domperidone (assay ≥ 99.78%) was purchased from Lee Pharma Ltd., India. Venlafaxine hydrochloride (assay ≥ 99.83%) was purchased from Aarti Industries Ltd., India. Deionized ultrapure water (18.2 MΩ cm at 25 ºC) was obtained using Milli-Q water purification system (Milli-Q Plus, Millipore®, France). Potassium dihydrogen phosphate, disodium hydrogen phosphate, glycine, sodium hydroxide and sodium chloride were purchased from S.D. Fine Chemical Ltd., India. Hydrochloric acid (fuming 37%) and orthophosphoric acid (Assay ≥ 85%) were purchased from Merck Chemical Ltd., India. All chemicals were of analytical grade and used as received without any modification.

4.2.2. Equipment/Instruments

Digital analytical balances with ± 0.01 mg sensitivity (AG135, Mettler Toledo, Switzerland and TB-215D, Denver Instrument, Germany) were used for all weighing

purposes. The pH was determined with digital pH meter (pHTestr 30, Eutech Instruments, USA) having ± 0.01 pH sensitivity which equipped with a combined glass electrode and automatic thermal compensation probe. Hot air oven (MSW-211, Macro Scientific Woks, India) was used for drying purpose. Vortex shaker (SPINIX MC-01, Tarson, India) and ultrasonic bath sonicator (Toshiba, India) were used for mixing purpose. Orbital shaking incubator (MSW-132, Macro Scientific Woks, India); and refrigerated microcentrifuge (Centrifuge 5430 R, Eppendorf, Germany) were used for solubility analysis. Stability chamber (WIL-195, Wadegati Labequip Ltd., India) and humidity chamber (Thermolab Scientific Equipments Pvt. Ltd., India) were used to maintain ambient (25 ± 2 ºC/60 ± 5% RH) and accelerated temperature (40 ± 2 ºC/75 ± 5% RH) condition. Frost free refrigerator (RT42K5468SL, Samsung Group, South Korea) was used for refrigerated storage.

Thermal analysis was performed using heat-flux differential scanning calorimeter (DSC-60 Plus, Shimadzu Corporation, Japan) with TA-60WS thermal analyzer and FC-60A flow controller (0 to 200 ml min^{-1} nitrogen gas), connected to a computer loaded with TA-60 software (version 1.51). It has temperature program rate from 0 to 99 ºC min^{-1}. The instrument can operate over temperature range of -140 to 600 ºC with heat flow range of ± 150 Mw.

IR spectra were recorded using Fourier transform infrared (FTIR) spectrophotometer with attenuated total reflectance (ATR) technique (IRAffinity-1S, Shimadzu Corporation, Japan) connected to computer loaded with LabSolutions IR Software (version 2.10). It can be operated over wavenumber range of 7800 to 350 cm^{-1} with a maximum resolution of 0.5 cm^{-1}. All equipment used for estimation of the drug were described in chapter 3.

4.2.3. Bulk characterization

Domperidone and venlafaxine hydrochloride are official in many countries and listed in various pharmacopoeias. However, few in-house tests were performed to establish identification and characterization of domperidone and venlafaxine hydrochloride.

4.2.3.1. Assay

For assay and percent purity purpose, in-house developed UV spectroscopic method was used as described in Chapter 3.

4.2.3.2. Spectral analysis

a. Ultraviolet absorption spectra

25 µg ml^{-1} solutions of domperidone and venlafaxine hydrochloride were prepared in 100 mM HCl and deionized water respectively and ultraviolet (UV) spectra were recorded in the range of 400 to 200 nm using UV spectrophotometer.

b. Infrared absorption spectra

For recording IR spectrum, FTIR with attenuated total reflectance (ATR) technique was used. Drug sample was placed on ATR crystal plate (zinc selenide crystal). The infrared absorption spectra were recorded in the range of 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} using high energy ceramic discharge metal halide lamp as light source and a temperature controlled DLaTGS (deuterated lanthanum α-alanine doped tri-glycine sulphate) detector. Recorded IR spectra were compared with that of the standard.

4.2.3.3. Thermal analysis

Thermal behavior of the pure domperidone and venlafaxine hydrochloride were studied using differential scanning calorimeter (DSC). To maintain a uniform temperature in the furnace and intracooler as well as to operate under steady nitrogen flow, DSC instrument was turned on 25 min prior to analysis. The instrument was calibrated using a pure indium (melting point 156.6 ºC) and zinc (melting point 419.5 ºC) as a calibration standard. Around 2 mg of finely pulverized drug samples were taken and sealed in a non-hermetic aluminum pan with a lid and placed test holder, while an empty sealed pan was used as a reference. Considering the melting point of drug samples, DSC thermogram was recorded over a temperature range of 30 to 350 ºC at 10 ºC min^{-1} heating rate. The inert environment was maintained by purging nitrogen gas at 40 ml min^{-1} flow rate.

4.2.4. Solubility analysis

Number of methods are available for the estimation of solubility of a drug which can be classified into two groups viz. kinetic solubility methods and thermodynamic solubility method. Kinetic solubility methods, such as direct UV analysis, nephelometry and turbidometry methods, are generally used in drug discovery as these are high throughput methods. Thermodynamic solubility is mainly determined using equilibrium shake flask method and potentiometric method. These methods are commonly used for a drug candidate in clinical studies [13]. Also, many commercial software products such as

QikProp, SLIPPER, CSlogWS, etc. can predict the solubility of a compound based on its structure [14].

The solubility studies for domperidone and venlafaxine hydrochloride in different pH and distilled water were determined using modified equilibrium shake flask method [13,15,16]. Buffered and unbuffered solutions of pH ranging from 1 to 12 were prepared. 10 ml of these solutions were transferred to 15 ml centrifuge tubes in which excess of drug was added and kept for shaking in an orbital shaking incubator at 37 ± 1 ºC. Centrifuge tubes containing drug samples were taken after 24 hr and centrifuged for 20 min at 15000 rpm at 25 ºC and supernatant was collected which was further filtered and analyzed with UV-spectroscopic method after appropriate dilution.

4.2.5. Stability studies

4.2.5.1. Solution state stability

Solution state stability of domperidone and venlafaxine hydrochloride was established in different buffered solutions of pH 1.2, 2, 3, 4, 5, 6, 7, 8, 9 and 10. A stock solution of 100 µg ml^{-1} domperidone was prepared in phosphate buffer of pH 3 and spiked in various buffer solutions to get a final concentration of 500 ng ml^{-1}. On the other hand, a stock solution of 1 mg ml^{-1} venlafaxine hydrochloride was prepared in water and spiked in various buffer solutions to get a final concentration of 1000 ng ml^{-1}. The prepared samples were stored in glass vials at 25 ± 2 ºC. The samples were withdrawn at different time points (0, 2, 4, 6, 8, 10, 12, 24, 36, 48 and 72 hr) and analyzed using in-house developed stability indicating HPLC methods. The amount of drug remaining in solution was plotted as a function of time. The results were analyzed to determine the order of degradation, degradation rate constants (K_{deg}) and time taken for 10% of degradation ($t_{90\%}$).

4.2.5.2. Solid state stability

To evaluate the solid state stability, both drug samples were stored at different storage conditions such as long term stability condition or controlled room temperature (CRT: 25 ± 2 ºC / 60 ± 5% RH), accelerated condition (AT: 40 ± 2 ºC / 75 ± 5% RH) and refrigerated condition (FT: 5 ± 2 ºC) as per ICH and WHO stability testing guidelines [17,18]. At predetermined time intervals (1, 2, 3, 4, 5 and 6 months), samples were withdrawn and characterized for physical observations and drug content [19,20]. The drug content in the samples was analyzed using validated stability indicating HPLC

methods (Chapter 3). All samples were analyzed in triplicate. Finally, results were analyzed to determine the order of degradation, K_{deg} and $t_{90\%}$.

4.2.6. Drug-excipient compatibility study

Investigating compatibility of the drug with various excipients is one of the critical tasks of preformulation study. Currently, there are no guidelines or universally accepted protocols available for drug-excipient compatibility studies [21]. Number of techniques such as DSC, isothermal microcalorimetry, hot stage microscopy, FTIR, X-ray diffraction method (XRD), solid state nuclear magnetic resonance spectroscopy (ssNMR), scanning electron microscopy, etc. have been used by researchers to investigate drug-excipient compatibility [22-28].

In this study, the compatibility of domperidone and venlafaxine hydrochloride were investigated using DSC, isothermal stress testing (IST) and FTIR techniques [29,30]. Binary mixtures of the drug with each selected excipient (1:1, w/w) were prepared by geometric mixing. All admixtures were passed through sieve of 250 µm aperture (#60) to ensure the uniform mixing. Excipients selected include Carbopol 971P, Carbopol 974P, microcrystalline cellulose (Avicel PH101), lactose, starch, di-calcium phosphate (DCP), and talc. All samples were analysed using DSC immediately after preparation of binary mixtures. For isothermal stress testing, all these admixtures were stored at 25 ± 2 ºC / 60 ± 5% RH and 40 ± 2 ºC / 75 ± 5% RH in both open and closed containers for 1 month. After 1 month, the drug was estimated using in house developed stability indicating HPLC method and FTIR studies were carried to check the interaction.

4.3. Results and discussion

4.3.1. Bulk characterization

4.3.1.1. Assay

The analyzed samples showed that domperidone and venlafaxine hydrochloride are 99.89% and 99.85% pure, respectively. Observed purity values were in good agreement with the labeled purity.

4.3.1.2. Spectral Analysis

a. **Ultraviolet absorption spectra**

The UV spectroscopic analysis of 25 µg ml^{-1} solutions of domperidone in 100 mM HCl showed maximum absorption at 284 nm while 25 µg ml^{-1} solutions of venlafaxine hydrochloride in deionized water showed maximum absorption at 224 nm. The UV

spectra of domperidone and venlafaxine hydrochloride are represented in Figure 4.1 and Figure 4.2, respectively.

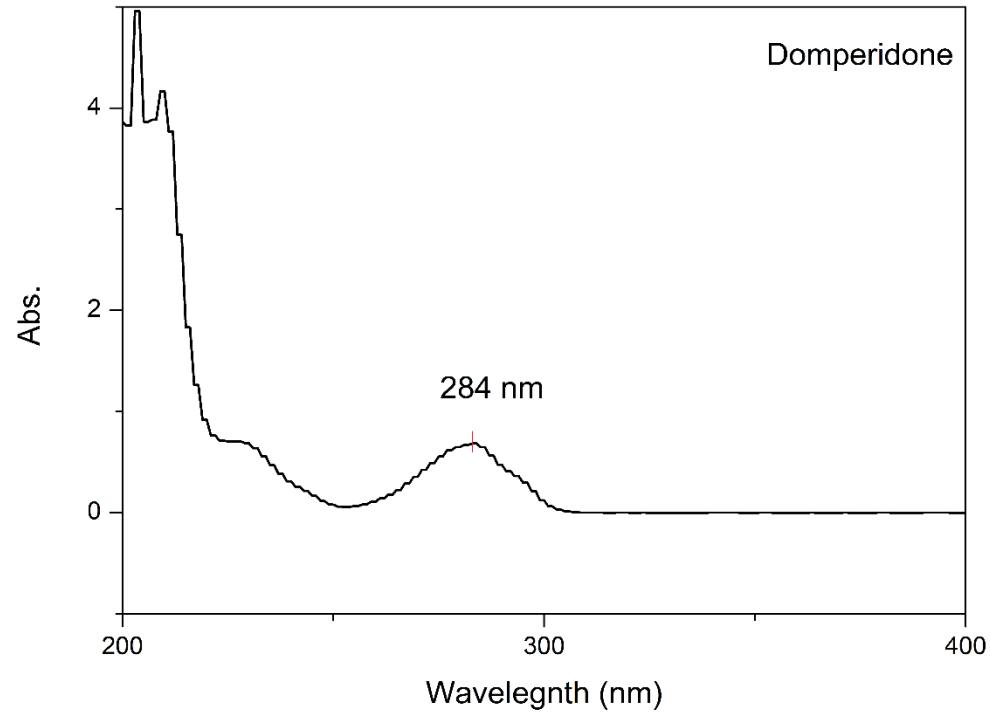

Figure 4.1. Representative UV spectra of domperidone (25 µg ml^{-1}) in 100 mM HCl

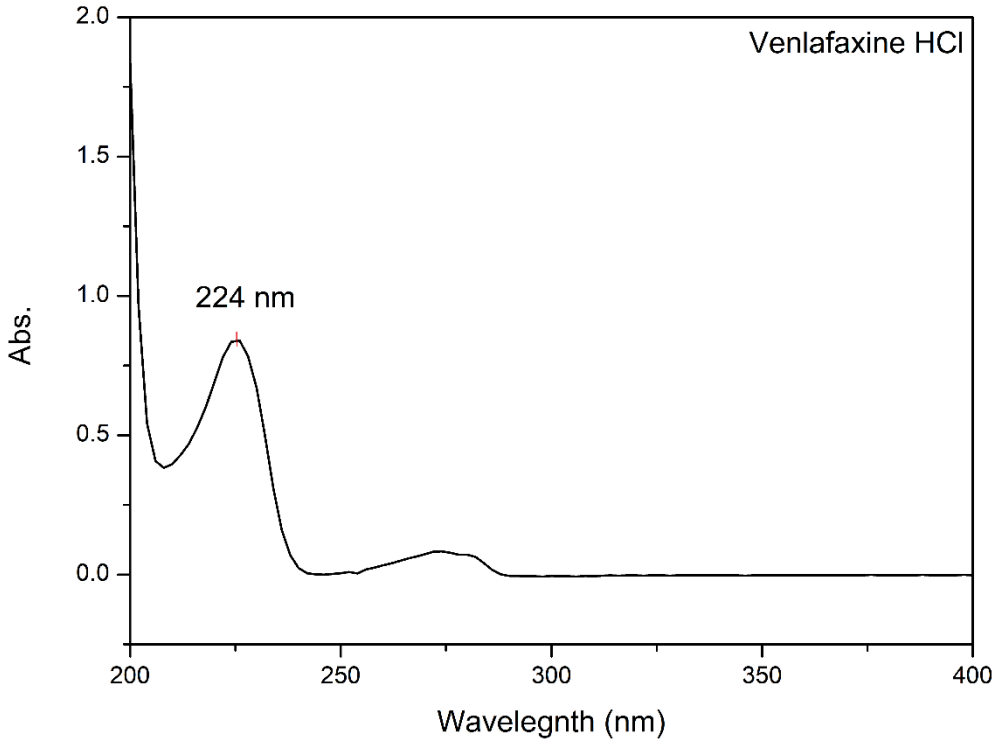

Figure 4.2. Representative UV spectra of venlafaxine hydrochloride (25 µg ml^{-1}) in deionized water

b. **Infrared absorption spectra**

The FTIR spectra of pure drugs demonstrated characteristic peaks which are in agreement with the reported data [31-33]. The FTIR spectrum of domperidone showed characteristic bands at 3093.82 & 3016.67 cm^{-1} (-NH stretching), 2931.80 & 2816.07 cm^{-1} (-CH stretching), 1689.84 and 1620.21 cm^{-1} (-C=O stretching), 1481.33 cm^{-1} (aromatic C=C bending), and 864.11 cm^{-1} (aromatic C-H bending). The representative FTIR spectrum for domperidone is shown in Figure 4.3.

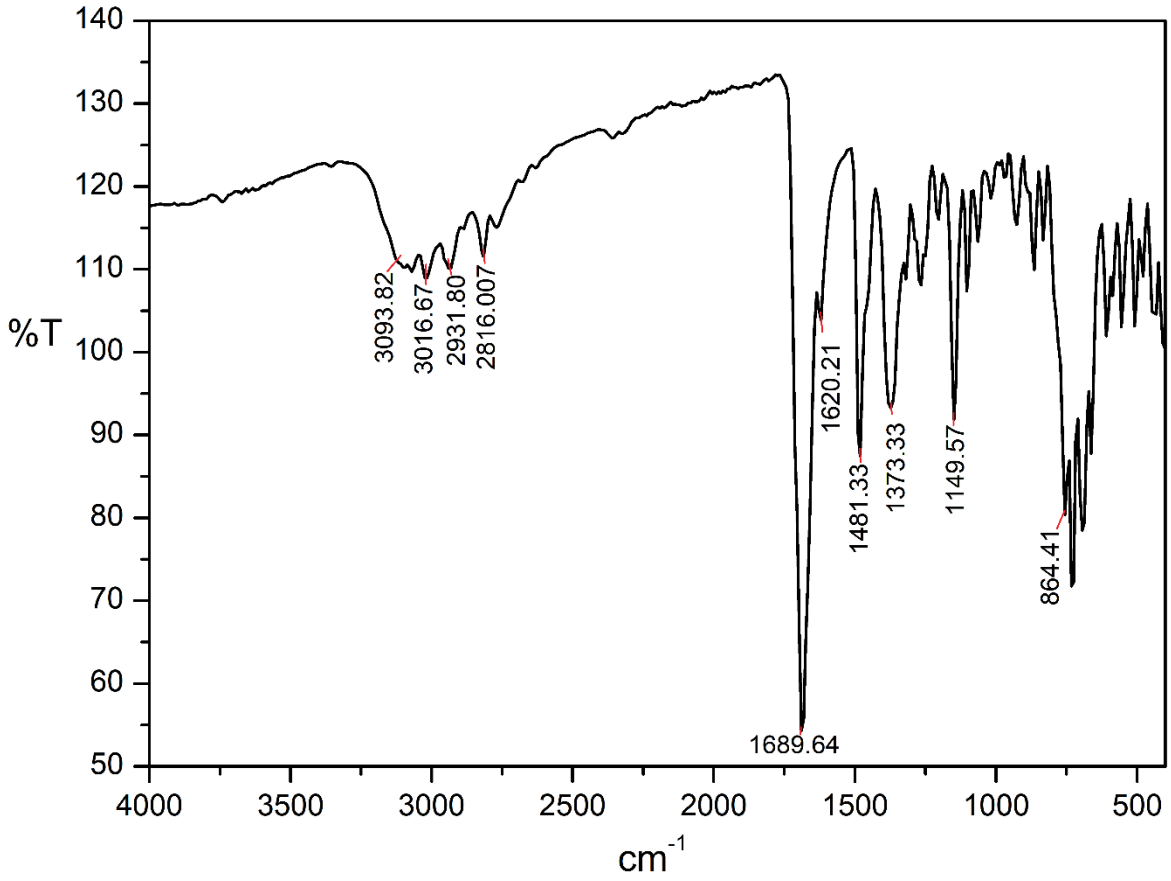

Figure 4.3. Representative FTIR spectrum of domperidone

The IR spectrum of venlafaxine showed characteristic bands at 3317.56 cm^{-1} (-OH stretching), 2939.52 cm^{-1} (-CH stretching), 1242.16 & 1620.21 cm^{-1} (-C-O stretching), 1465.90 cm^{-1} (alkane CH_2-), 1512.19 cm^{-1} (aromatic C=C bending), 1041.56 cm^{-1} (asymmetric C-O-C) and 825.53 cm^{-1} (-C-N bending). The representative FTIR spectrum for venlafaxine hydrochloride is shown in Figure 4.4.

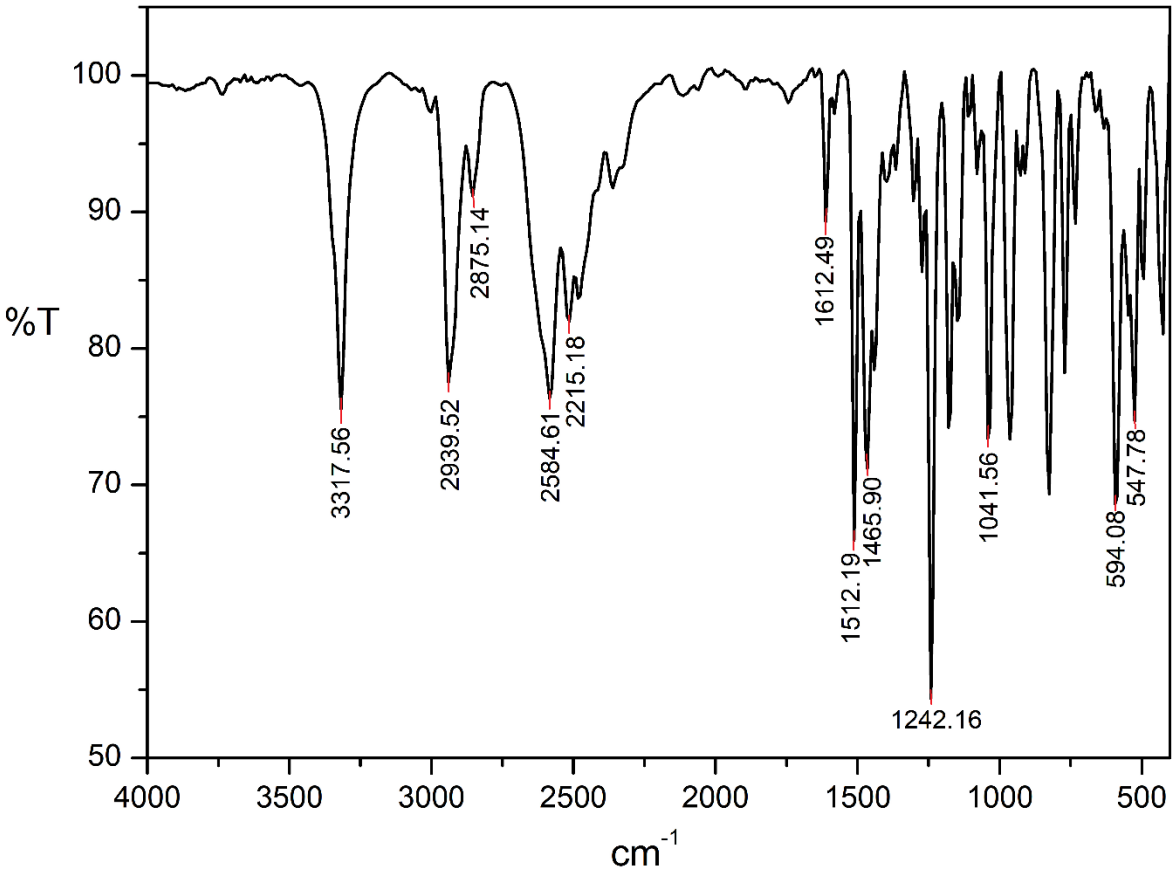

Figure 4.4. Representative FTIR spectrum of venlafaxine hydrochloride

4.3.2. Thermal analysis

The DSC thermogram of pure domperidone showed melting endotherm at 251.30 ºC and melting enthalpy (ΔH) of -110.18 J g^{-1}. Thermal curve of domperidone also indicated that drug was in a crystalline anhydrous state. The representative DSC thermogram for domperidone is shown in Figure 4.5. While DSC thermogram of venlafaxine hydrochloride showed sharp melting endotherm at 216.29 ºC and melting enthalpy (ΔH) of -33.92 J g^{-1}. The sharp endothermic peak of drug confirmed that drug has a crystalline nature. The representative DSC thermogram for venlafaxine hydrochloride is shown in Figure 4.6.

4.3.3. Solubility analysis

In solubility study at various pH, domperidone showed pH dependent solubility with higher solubility in acidic media and these results were in accordance with already reported studies [34]. Domperidone is a weakly basic drug with pKa of 7.9 which favors ionization at pH below 7.9. Thus, domperidone has higher solubility in acidic medium. The pH solubility profile of domperidone is represented in Figure 4.7.

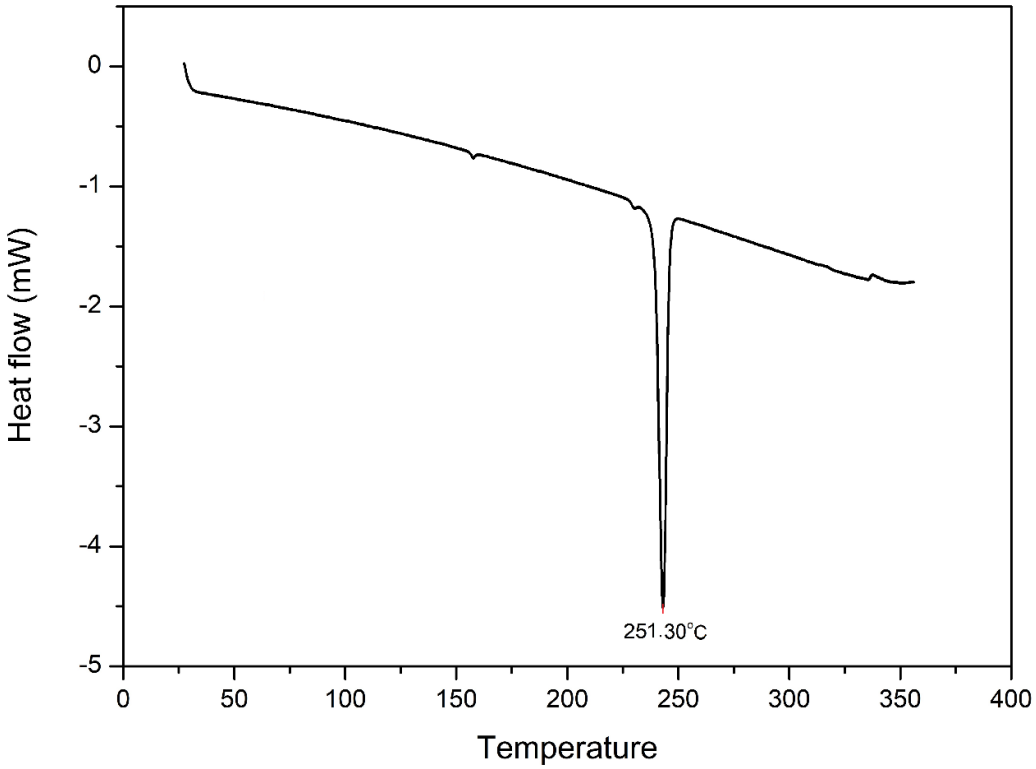

Figure 4.5. Representative DSC thermogram of pure domperidone

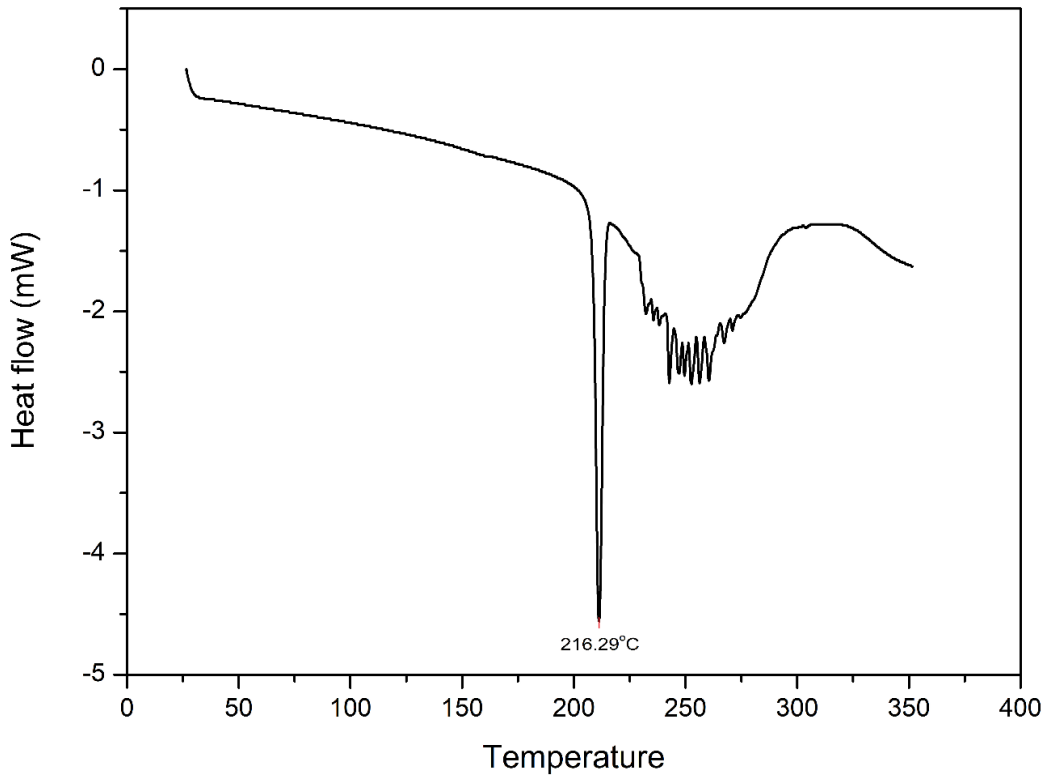

Figure 4.6. Representative DSC thermogram of pure venlafaxine hydrochloride

On the other hand, venlafaxine hydrochloride is a salt of a weak base and a strong acid with pKa of 9.6 (strongest acidic) and 14.0 (strongest basic). It showed higher solubility which was not affected by pH of the media. The pH solubility profile of venlafaxine hydrochloride is represented in Figure 4.8.

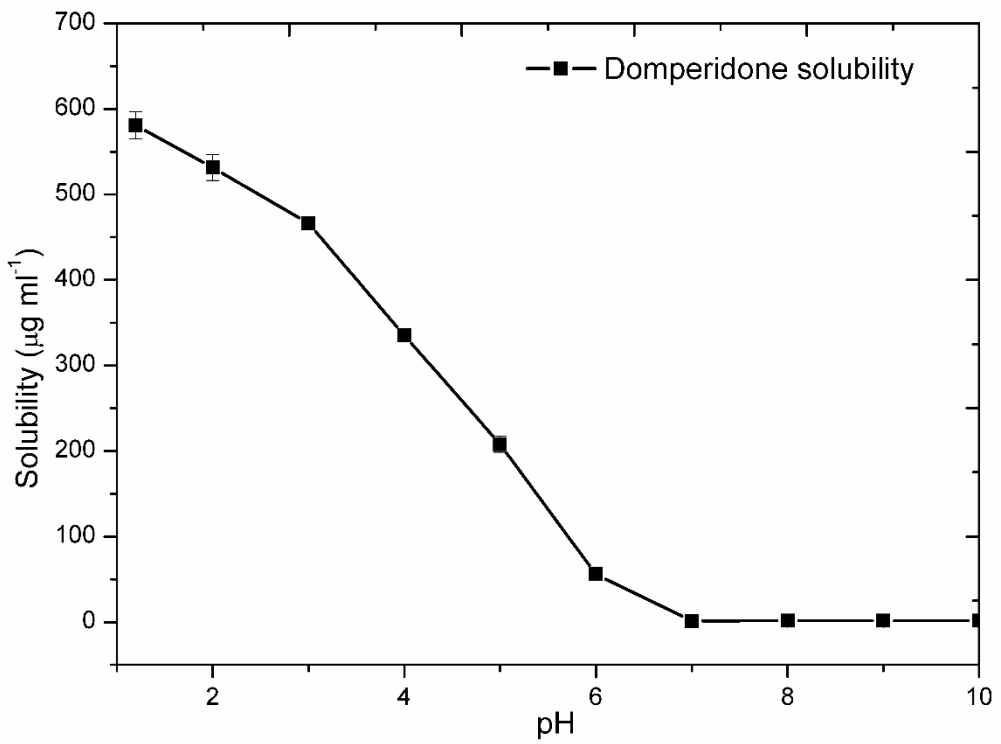

Figure 4.7. Solubility study: pH solubility profile of domperidone

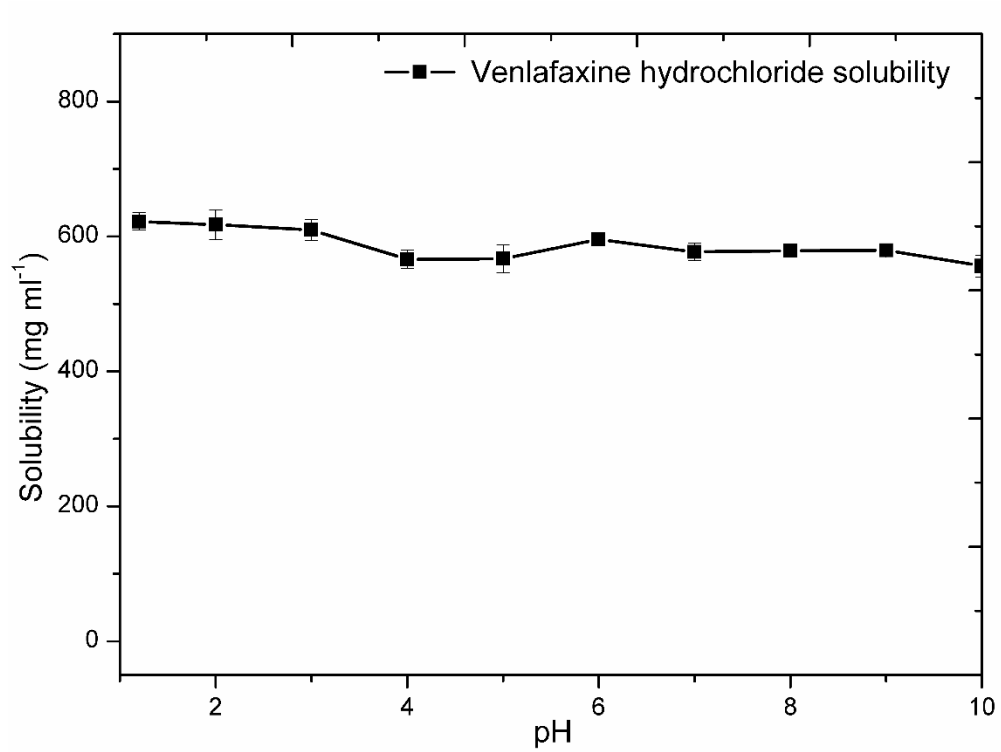

Figure 4.8. Solubility study: pH solubility profile of venlafaxine hydrochloride

4.3.4. Stability studies

4.3.4.1. Solution state stability

Solution state stability of domperidone and venlafaxine hydrochloride was determined in buffered solutions of pH ranging from 1.2 to 10. The log of percentage drug remaining to be degraded (%RTD) versus time profiles for domperidone and venlafaxine hydrochloride in various pH solutions were plotted and analyzed for K_{deg} and $t_{90\%}$ values. First order kinetics was observed for the degradation of both drugs which is evident by high regression coefficient values. Table 4.1 summarizes the K_{deg} and $t_{90\%}$ values obtained at various pH values. The K_{deg} values obtained were ranging from 1.34×10^{-4} to 1.78×10^{-4} hr^{-1} and 2.01×10^{-4} to 2.33×10^{-4} hr^{-1} for domperidone and venlafaxine hydrochloride respectively. The $t_{90\%}$ values obtained were ranging from 24.61 to 32.81 days and 19.69 to 21.97 days for domperidone and venlafaxine hydrochloride respectively.

Table 4.1. Solution state stability studies data

pH of media	Domperidone			Venlafaxine hydrochloride		
	K_{deg} (hr^{-1})	r^2	$t_{90\%}$ (days)	K_{deg} (hr^{-1})	r^2	$t_{90\%}$ (days)
1.2	0.000156	0.9233	28.12	0.000201	0.8793	21.87
2	0.000178	0.9632	24.61	0.000223	0.8654	19.69
3	0.000156	0.8920	28.12	0.000223	0.8925	19.69
4	0.000156	0.8774	28.12	0.000201	0.9591	21.87
5	0.000178	0.9647	24.61	0.000223	0.8953	19.69
6	0.000134	0.9644	32.81	0.000201	0.9364	21.87
7	0.000156	0.9002	28.12	0.000201	0.9201	21.87
8	0.000156	0.9009	28.12	0.000223	0.8813	19.69
9	0.000156	0.9626	28.12	0.000223	0.9127	19.69
10	0.000156	0.9128	28.12	0.000223	0.8520	19.69

4.3.4.2. Solid state stability

Solid state stability data revealed that both drugs followed first order degradation which is evident by high regression coefficient values. Table 4.2 summarizes the results of solid state stability studies. Domperidone and venlafaxine hydrochloride were found stable under refrigerated conditions, with $t_{90\%}$ values of 76.26 and 65.37 months respectively. In addition, both domperidone and venlafaxine hydrochloride were found to be stable at controlled room temperature with $t_{90\%}$ values of 65.36 and 57.20 months respectively.

Similarly, domperidone and venlafaxine hydrochloride also showed good stability at accelerated temperature with $t_{90\%}$ values of 28.59 and 24.08 months respectively. In addition, FTIR spectrum revealed that there is no change in the infrared absorption peaks at the end of 12 months. Moreover, thermal analysis confirmed that there is no evidence of any significant change in DSC thermogram of both drugs. Thus, DSC and FTIR studies indicate that both domperidone and venlafaxine hydrochloride maintained their physical and chemical form.

Table 4.2. Solid state stability studies data

Storage conditions	Domperidone			Venlafaxine hydrochloride		
	K_{deg} (month^{-1})	r^2	$t_{90\%}$ (month)	K_{deg} (month^{-1})	r^2	$t_{90\%}$ (month)
FT: 5 ± 2 ºC	0.001382	0.9094	76.26	0.001612	0.9380	65.37
RT: 25 ± 2 ºC /60 ± 5% RH	0.001612	0.9052	65.36	0.001842	0.8996	57.20
AT: 40 ± 2 ºC / 75 ± 5%	0.003685	0.9448	28.59	0.004376	0.9741	24.08

4.3.5. Drug-excipient compatibility study

Thermal analysis was carried out using DSC. Any abrupt or drastic change in DSC thermogram may indicate a possible drug-excipient interaction. The thermograms obtained for pure drug, pure excipients, binary mixtures of drug and excipients are represented in Figure 4.9 to 4.20. The melting endotherms of both domperidone and venlafaxine hydrochloride were well preserved in all the cases. Also, only slight shift in enthalpy was observed in few cases (Table 4.3). These observations clearly suggested the absence of incompatibility between selected excipients and both drugs.

In IST studies, in-house developed a stability indicating methods (described in Chapter 3) were used for the estimation of the drug in binary mixtures. Table 4.4 summarizes results for binary mixtures stored at 40 ± 2 ºC / 75 ± 5% RH in open containers. No loss was observed in any of the samples stored at 25 ± 2 ºC / 60 ± 5% RH and 40 ± 2 ºC / 75 ± 5% RH, indicating absence of incompatibility in selected excipients and both drugs. These results were further supported by FTIR study. In all binary mixtures of drug and excipients, all characteristic IR absorption peaks of both drugs were identified. The FTIR spectra of all binary mixtures are represented in Figure 4.21 and 4.22.

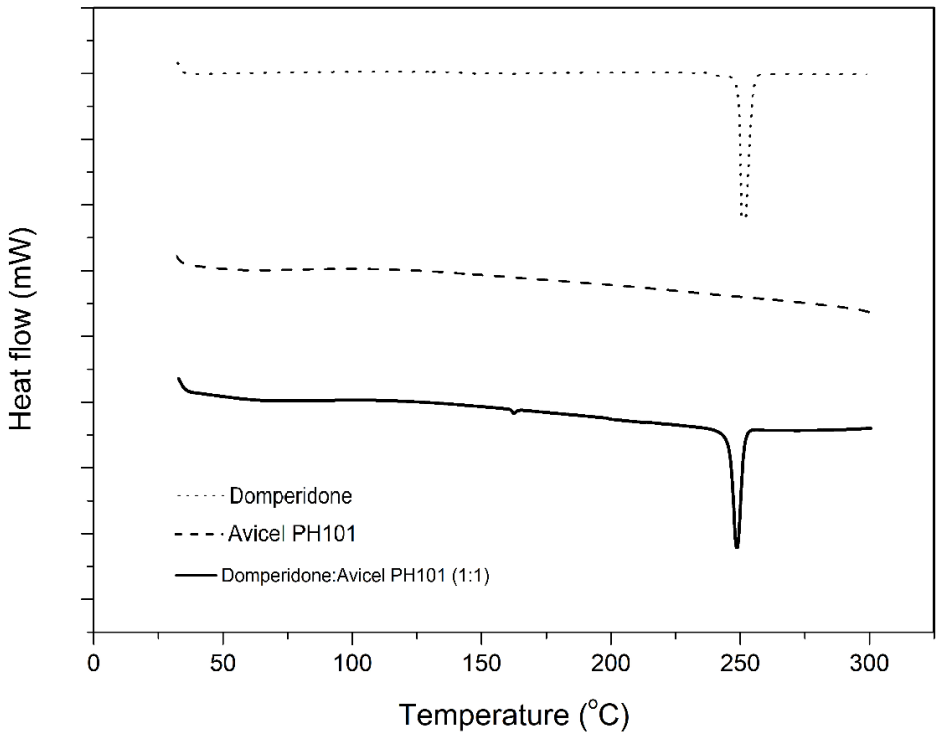

Figure 4.9. Overlaid DSC thermograms of pure domperidone, pure Avicel PH101 and physical mixture of domperidone and Avicel PH101 (1:1, w/w)

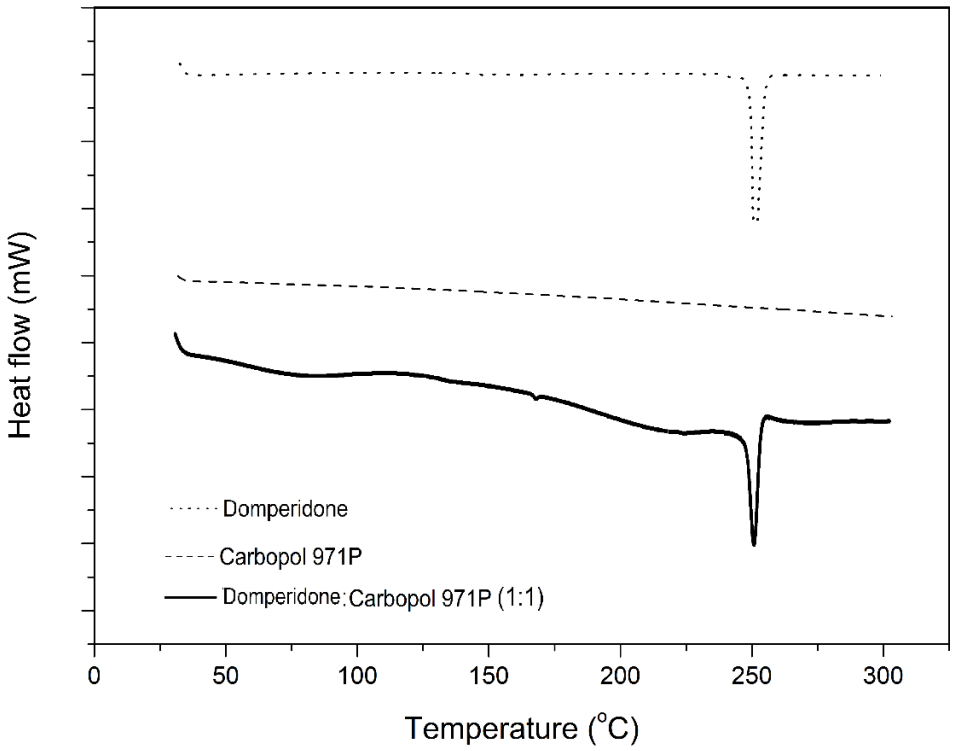

Figure 4.10. Overlaid DSC thermograms of pure domperidone, pure Carbopol 971 and physical mixture of domperidone and Carbopol 971 (1:1, w/w)

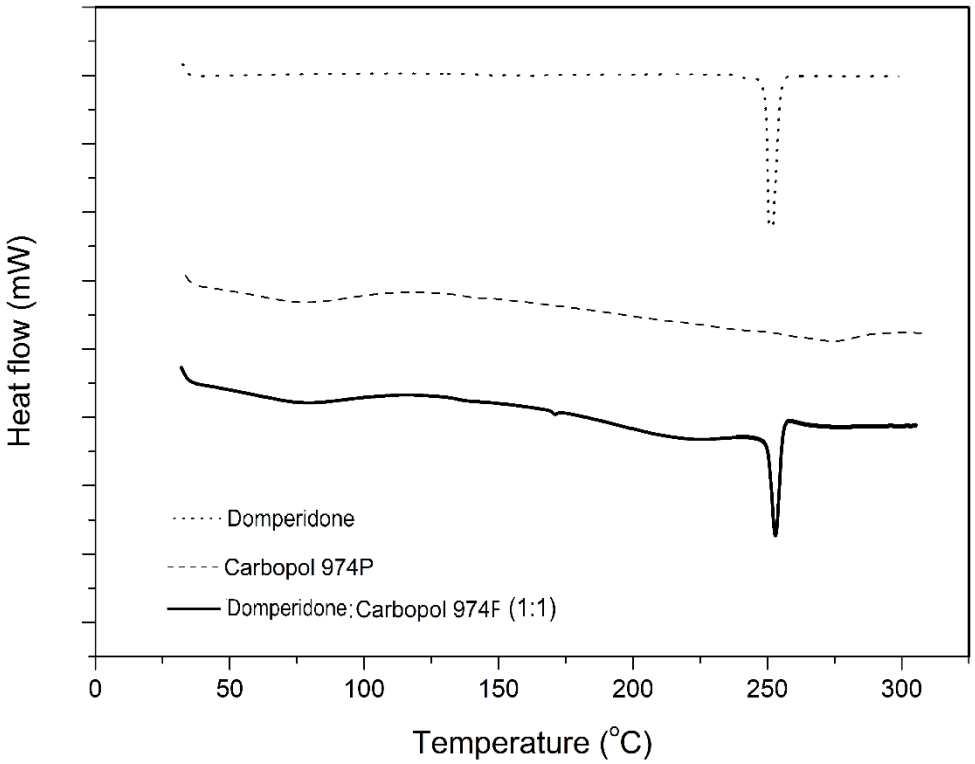

Figure 4.11. Overlaid DSC thermograms of pure domperidone, pure Carbopol 974 and physical mixture of domperidone and Carbopol 974 (1:1, w/w)

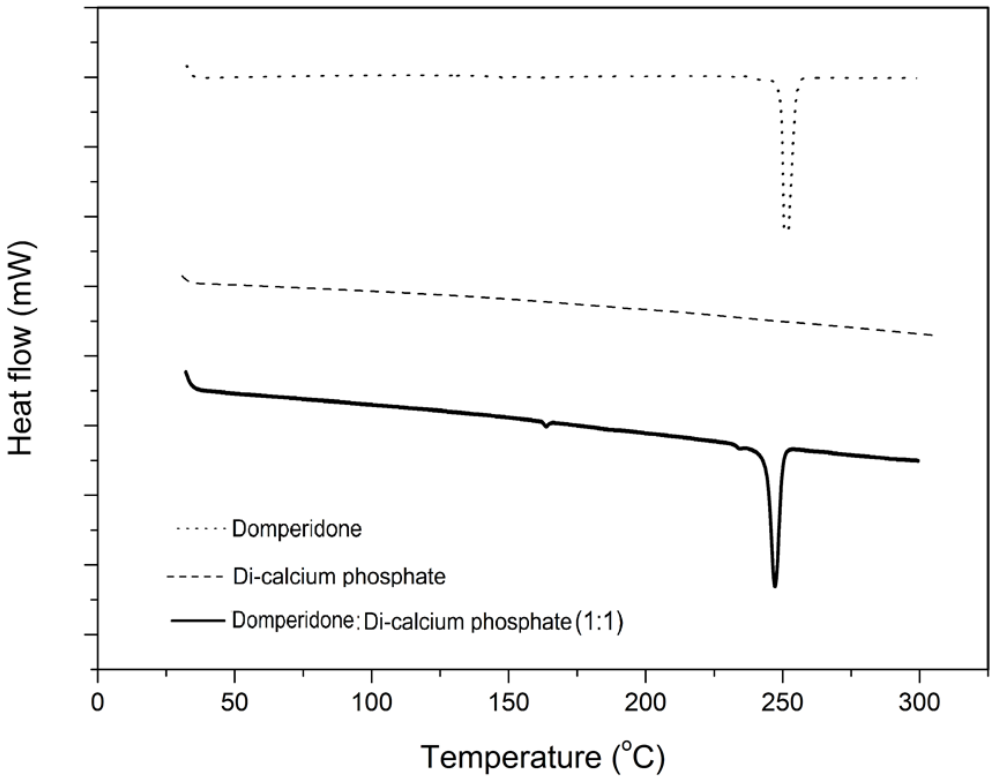

Figure 4.12. Overlaid DSC thermograms of pure domperidone, pure DCP and physical mixture of domperidone and DCP (1:1, w/w)

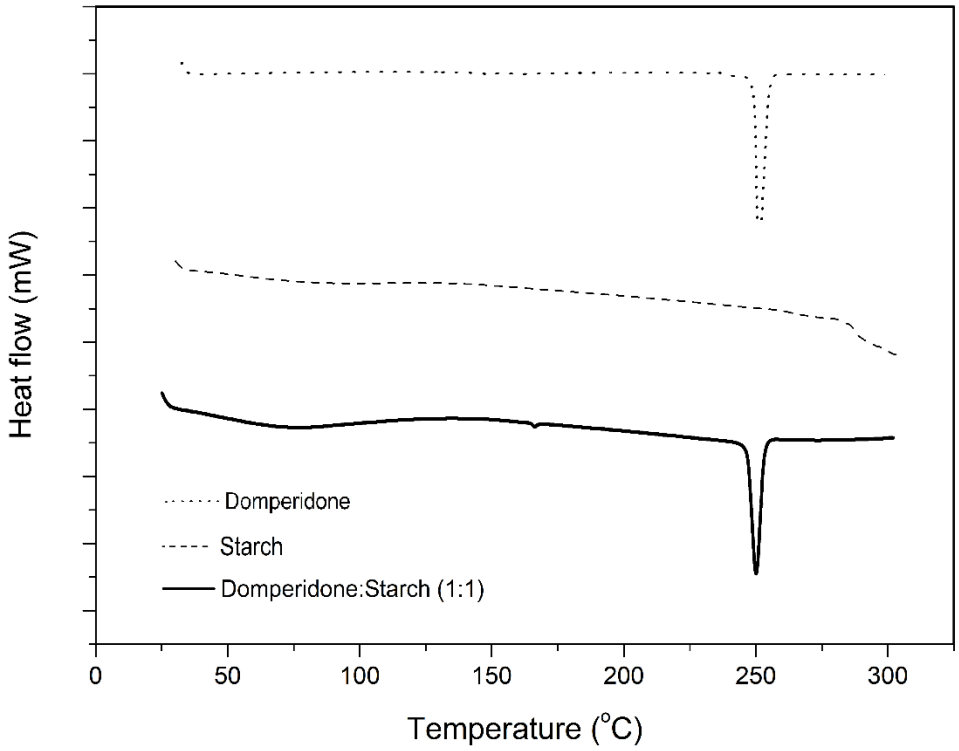

Figure 4.13. Overlaid DSC thermograms of pure domperidone, pure starch and physical mixture of domperidone and starch (1:1, w/w)

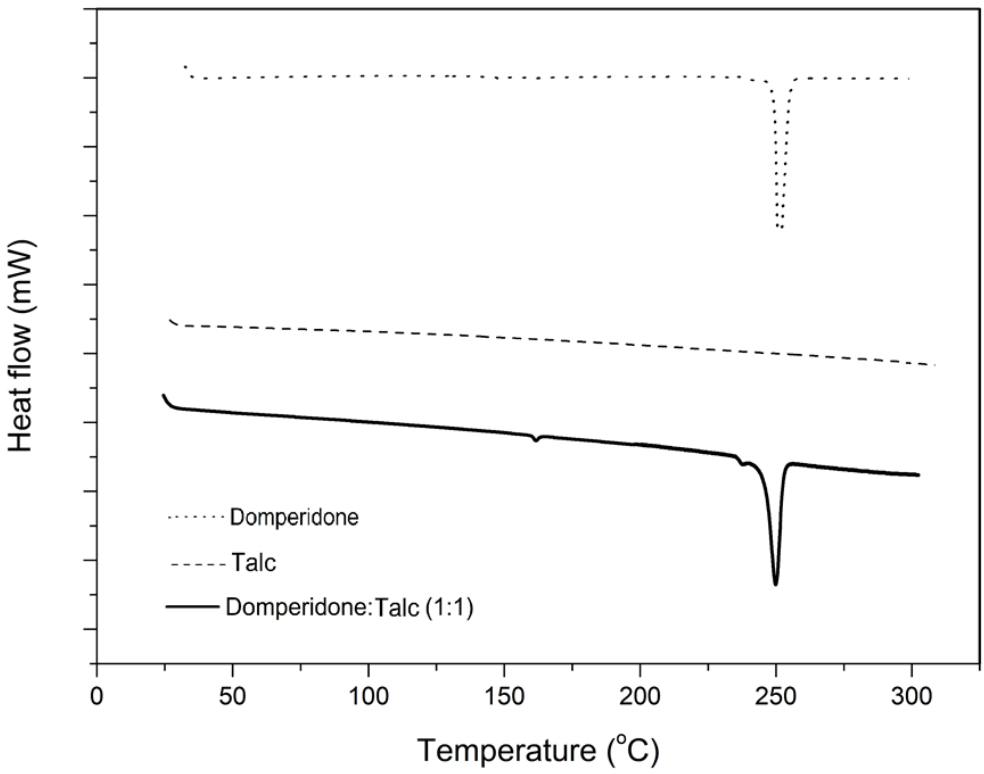

Figure 4.14. Overlaid DSC thermograms of pure domperidone, pure talc and physical mixture of domperidone and talc (1:1, w/w)

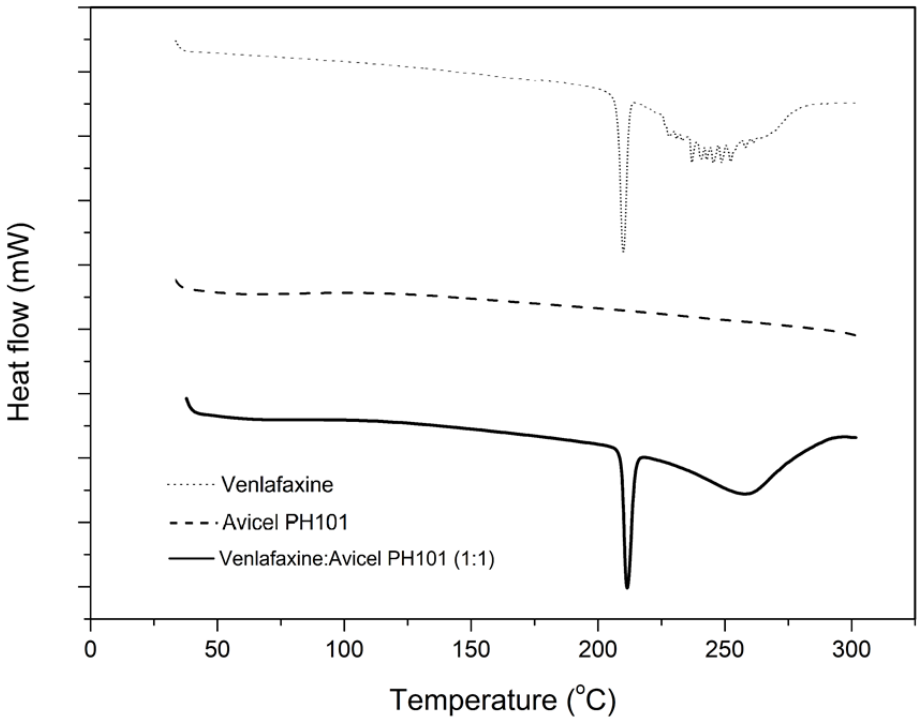

Figure 4.15. Overlaid DSC thermograms of venlafaxine, pure Avicel PH101 and physical mixture of venlafaxine and Avicel PH101 (1:1, w/w)

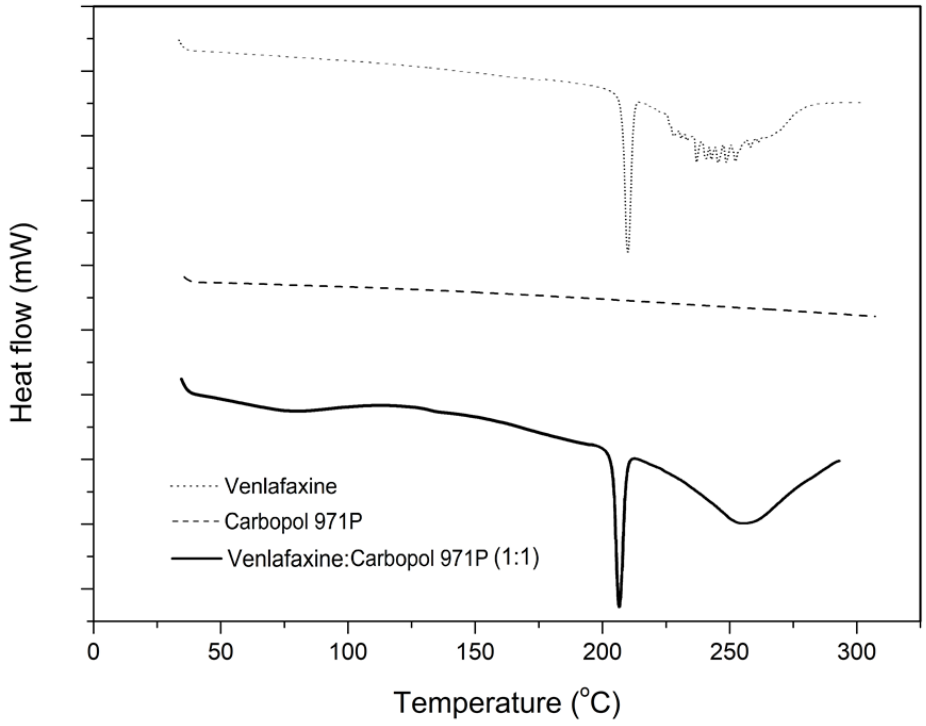

Figure 4.16. Overlaid DSC thermograms of pure venlafaxine, pure Carbopol 971 and physical mixture of venlafaxine and Carbopol 971 (1:1, w/w)

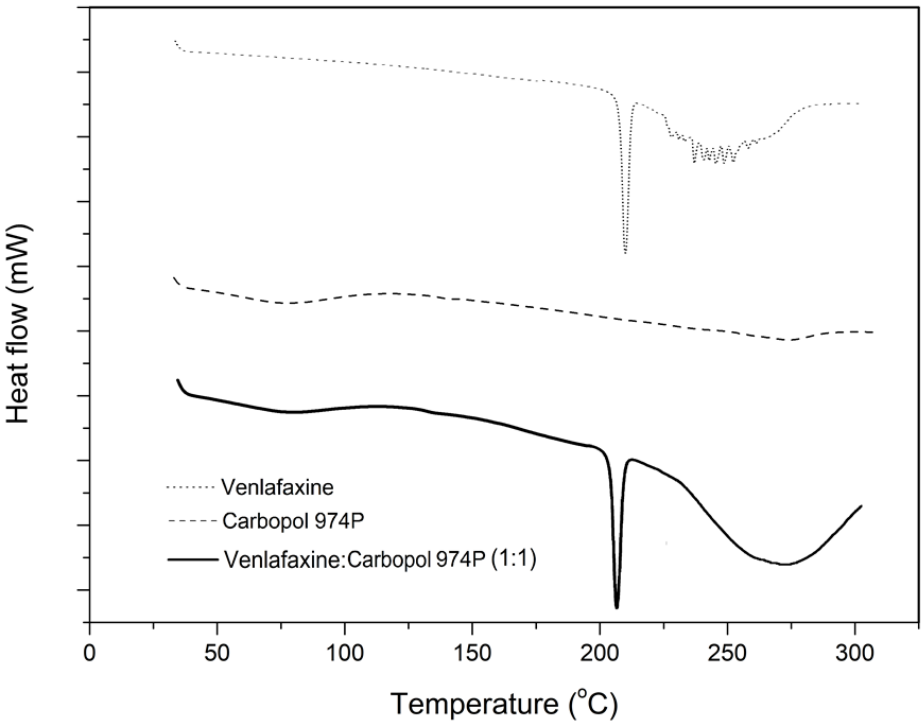

Figure 4.17. Overlaid DSC thermograms of pure venlafaxine, pure Carbopol 974 and physical mixture of venlafaxine and Carbopol 974 (1:1, w/w)

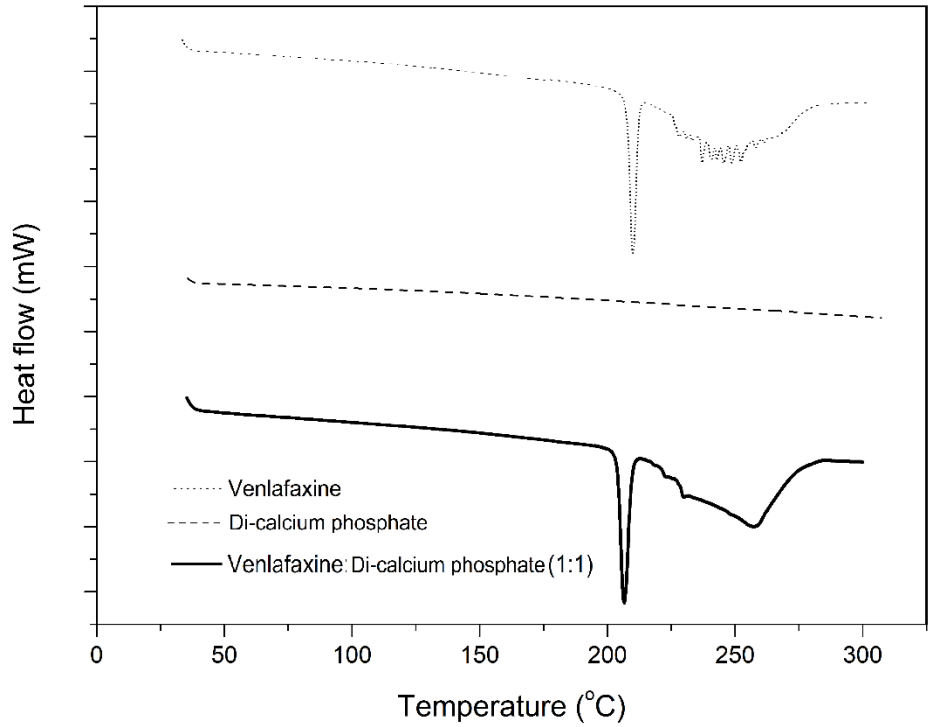

Figure 4.18. Overlaid DSC thermograms of pure venlafaxine, pure DCP and physical mixture of venlafaxine and DCP (1:1, w/w)

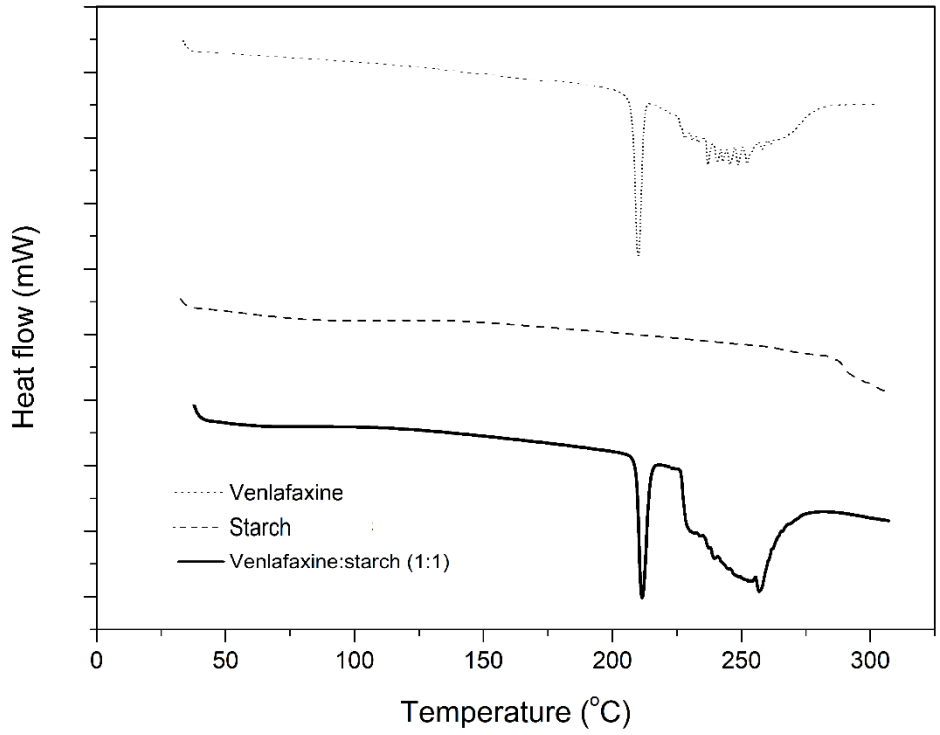

Figure 4.19. Overlaid DSC thermograms of pure venlafaxine, pure starch and physical mixture of venlafaxine and starch (1:1, w/w)

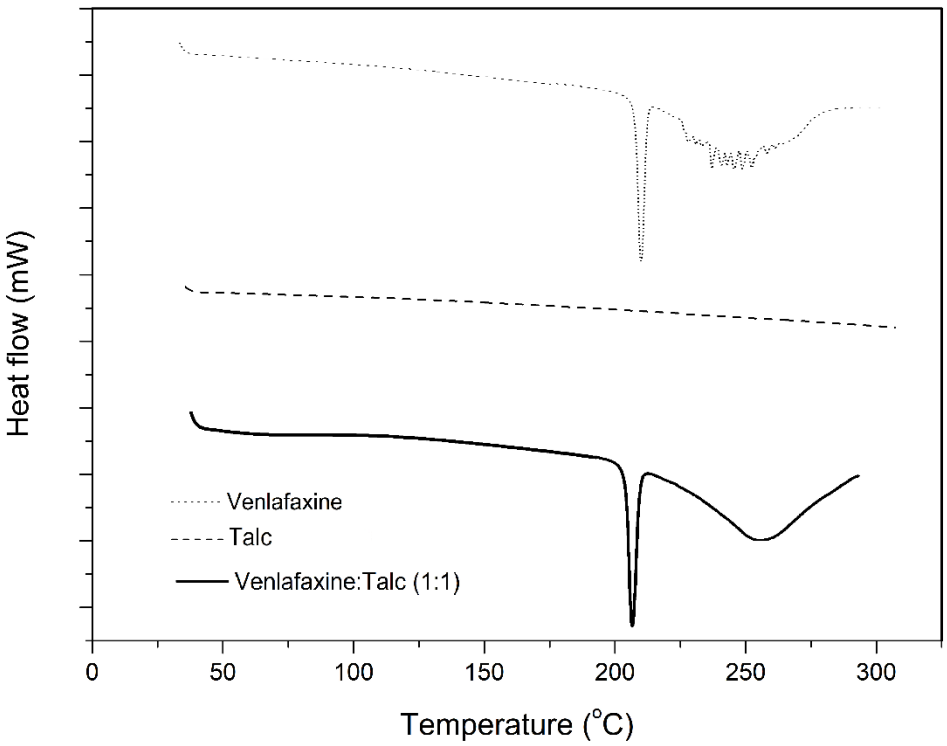

Figure 4.20. Overlaid DSC thermograms of pure venlafaxine, pure talc and physical mixture of venlafaxine and talc (1:1, w/w)

Table 4.3. Thermal analysis of drugs with selected excipients

Sample	Domperidone		Venlafaxine hydrochloride	
	DSC peak (°C)	Enthalpy (J g^{-1})	DSC peak (°C)	Enthalpy (J g^{-1})
Pure drug	251.30	-110.18	216.29	-33.92
Drug + Avicel PH101	251.40	-113.87	216.34	-33.88
Drug + Carbopol 971	250.38	-112.18	216.45	-34.18
Drug + Carbopol 974	251.60	-114.67	215.89	-33.12
Drug + DCP	250.90	-109.56	216.54	-34.45
Drug + Starch	251.34	-111.56	216.90	-35.58
Drug + Talc	251.48	-114.78	216.56	-34.55

Table 4.4. Drug-excipient compatibility studies (IST data)*

Sample	Domperidone		Venlafaxine hydrochloride	
	Assay ± SD[a] (% w/w)	Degradant (% w/w)	Assay ± SD[a] (% w/w)	Degradant (% w/w)
Pure drug	98.34 ± 0.35	Not observed	98.16 ± 0.53	Not observed
Drug + Avicel PH101	98.24 ± 0.63	Not observed	98.88 ± 1.27	Not observed
Drug + Carbopol 971	98.54 ± 0.04	Not observed	98.99 ± 0.45	Not observed
Drug + Carbopol 974	98.17 ± 0.15	Not observed	98.68 ± 1.52	Not observed
Drug + DCP	98.29 ± 0.26	Not observed	99.17 ± 0.49	Not observed
Drug + Starch	98.99 ± 0.45	Not observed	98.89 ± 0.90	Not observed
Drug + Talc	98.41 ± 0.18	Not observed	99.4 ± 0.43	Not observed

***for storage conditions: at 40 ± 2 °C / 75 ± 5% RH in open containers for 1 month**
[a] each value is average of three separate determination

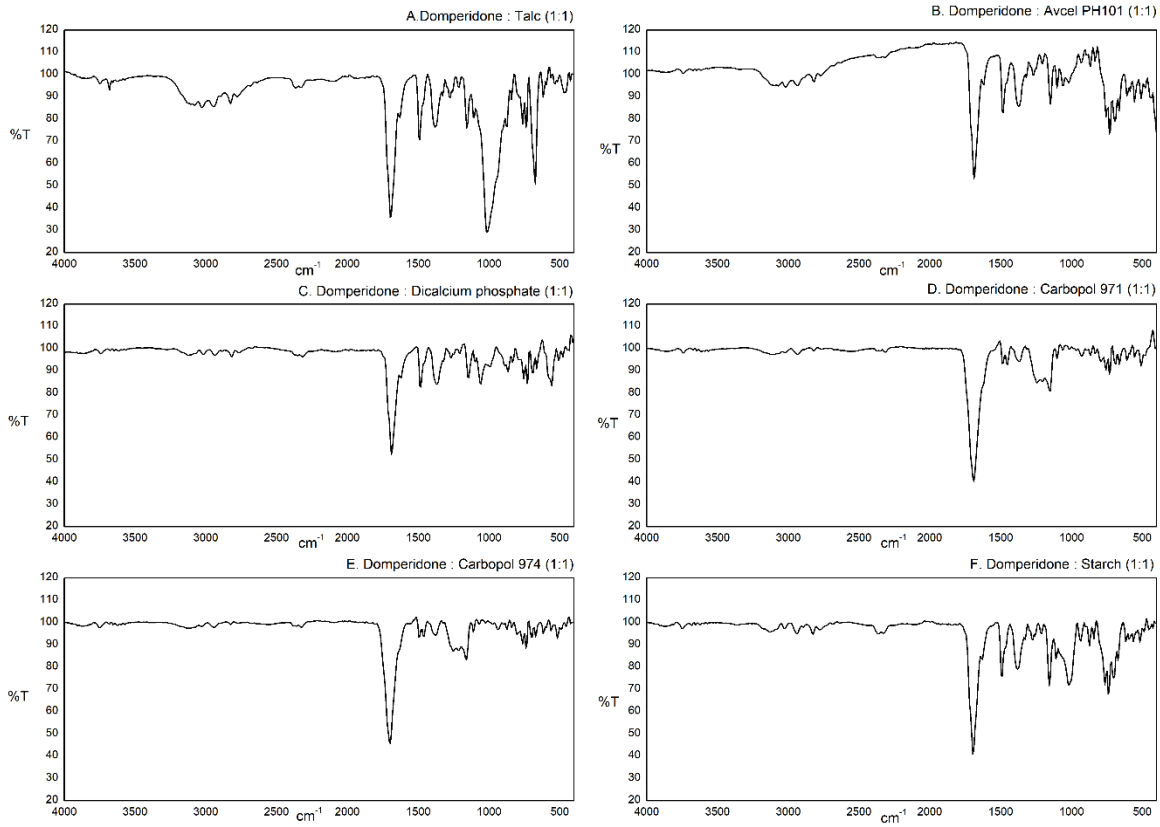

Figure 4.21. Drug-excipient compatibility for domperidone: FTIR study

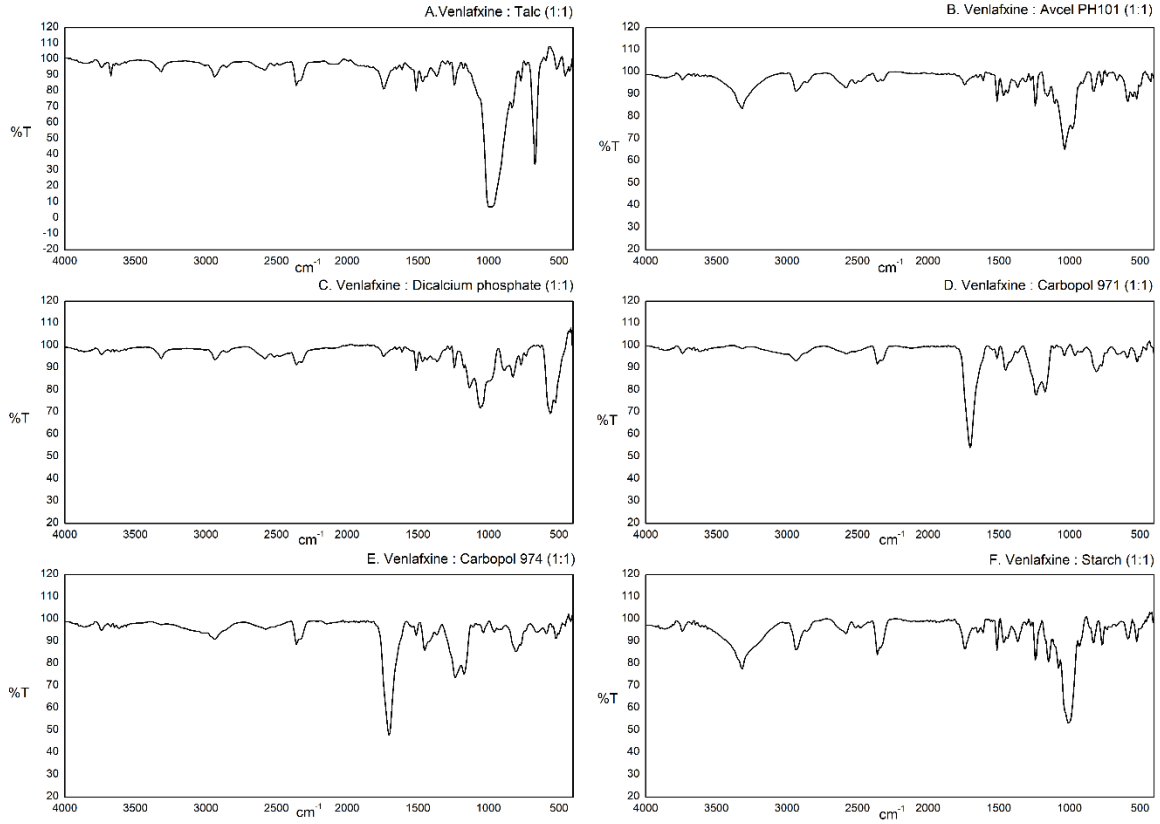

Figure 4.22. Drug excipient-compatibility for venlafaxine hydrochloride: FTIR study

4.4. Conclusion

Both domperidone and venlafaxine hydrochloride, procured from Lubrizol, exhibited satisfactory results in the assay and various identification tests carried out according to United State Pharmacopeia (USP) and Indian Pharmacopeia (IP). Solubility studies demonstrated that domperidone exhibited a pH dependent solubility having higher solubility in acidic pH buffers and low solubility in basic pH buffers. On the other hand, venlafaxine hydrochloride exhibited pH independent solubility. Solution state stability studies suggested that both domperidone and venlafaxine hydrochloride followed first order degradation and were found to be stable in both acidic and basic pH conditions during entire study interval (72 hr). The solid state stability studies confirmed that both drugs are stable at refrigerated and ambient temperature. Drug-excipient compatibility studies by thermal analysis revealed no significant interaction with various excipients selected for the formulation development. It was further confirmed by IST and FTIR studies. Thus, drug-excipient compatibility studies would support the rational for the selection of various excipients for the development of stable formulations and stability studies provided valuable information for deciding various storage conditions. Also, solubility study data would help in formulation design and selection of dissolution media for developed formulations. Therefore, these preformulation studies provided valued information for the development of formulations.

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5. Formulation Design and Development

5.1. Introduction

Carbopol polymers are high molecular weight, cross-linked, acrylic acid based polymers [1]. Currently, 11 grades of Carbopol polymers are available in the market and each grade has its distinctive significance in the preparation of pharmaceutical dosage form. Among these available Carbopol grades, Carbopol 971P, Carbopol 71G, Carbopol 974P and Noveon AA-1 are used in oral formulations [2]. However, Noveon AA-1 is commonly used in bioadhesive formulations and Carbopol 71G is granular grade of Carbopol 971P which is commonly used in the preparation of directly compressible tablets [3-4]. Thus, for our present study, Carbopol 971P and Carbopol 974P were selected. These oral grades of Carbopols are highly efficient release retarding agent and in many studies demonstrated slower release at lower amount than other commercially available hydrophilic release retarding polymers such as hydroxypropyl methylcellulose (HPMC), hydroxypropyl cellulose (HPC) and xanthan gum [5]. Owing to this outstanding release retarding properties of Carbopols, use of these polymers may allow us to use extrusion-spheronization technology to prepare matrix pellets. These controlled release (CR) matrix pellets prepared with extrusion-spheronization technology offers various benefits over currently used technologies viz. powder layering and fluidized bed processor, such as ease of preparation, operator independent process, nonrequirement of organic solvent, lower manufacturing time and cost effective [6-8]. As discussed earlier, the use of Carbopol polymers in this technology has not been fully studied and explored.

Solubility of drug has substantial impact on designing CR formulation as well as extrusion-spheronization process. Therefore, to study the wide range applicability of Carbopols, low soluble (domperidone) and high soluble (venlafaxine hydrochloride) drug were selected as model drugs. Thus, in this chapter, studies involving development of Carbopol based pellets of domperidone and venlafaxine hydrochloride using extrusion-spheronization technology have been presented. Further, the effect of process and formulation variables involved in extrusion-spheronization technology which affect the final pellet qualities such as amount of granulation fluid, amount of drug, screen aperture diameter, extruder speed, spheronization speed, spheronization time, spheronization load, drying technique and temperature were studied. Also, various quality control tests were performed for evaluation of developed pellet formulations. Further, batch reproducibility studies for developed pellets formulations were also

performed. Additionally, stability of developed pellet formulations was assessed at various conditions of temperature and humidity.

5.2. Experimental

5.2.1. Material

Domperidone (assay ≥ 99.78%) was purchased from Lee Pharma Ltd., India. Venlafaxine hydrochloride (assay ≥ 99.83%) was purchased from Aarti Industries Ltd., India. Microcrystalline cellulose (Avicel PH101 and Avicel PH102) was purchased from FMC BioPolymer Pvt. Ltd., USA. Carbopol 971P and Carbopol 974P were obtained as gift sample from Lubrizol Advanced Material Pvt. Ltd., India. Starch, talc and dicalcium phosphate (DCP) were purchased from Central Drug House Pvt. Ltd., India. Potassium dihydrogen phosphate, disodium hydrogen phosphate and sodium hydroxide were purchased from S.D. Fine Chemicals Ltd., India. Hydrochloric acid (fuming 37%) and orthophosphoric acid (Assay ≥ 85%) were purchased from Merck Chemicals Ltd., India. HPLC grade acetonitrile (assay > 99.7%) was also purchased from Merck Chemicals Ltd., India. Deionized ultrapure water (18.2 MΩ cm at 25 ℃) was obtained using Milli-Q water purification system (Milli-Q Plus, Millipore®, France). All chemicals were used as received without any modification.

5.2.2. Instrument/Equipments

Digital analytical balances (AG135, Mettler Toledo Pvt. Ltd., Switzerland; TB-215D, Denver Instruments, USA and BrAS 224, BR Biochem Life Sciences Pvt. Ltd., India) were used for all weighing purposes. The pH was determined with digital pH meter having ± 0.01 pH sensitivity (pHTestr 30, Eutech Instruments, USA) equipped with a combined glass electrode and automatic thermal compensation probe. Spinix vortex shaker (SPINIX MC-01, Tarson Products Pvt. Ltd., India), mechanical stirrer (RQ-124A/D, Remi Elektrotechnik Ltd., India) and ultrasonic bath sonicator (Toshiba Instruments Pvt. Ltd., India) were used for mixing purpose.

Planetary mixer (KM400, Kenwood mixer, Kenwood Ltd., UK) was used for dry mixing and wet mixing. For pellet preparation, single screw dome extruder and spheronizer with cross-hatch pattern plate (Sunsai Ltd., India) was used. Tray dryer (CM Envirosystems Ltd., India) was used for drying of pellets. In comparative study, for effect of drying techniques on the release profile of pellets, fluidized bed processor (Minilab Bottom Spray Coater, Umang Pharmatech Ltd., India) was also used for drying of pellets.

Sieve analysis of pellets was carried out using electromagnetic sieve shaker (EMS-8, Electrolab India Pvt. Ltd., India). Tapped density was evaluated using tap density tester (ETD-1020, Electrolab India Pvt. Ltd., India). Friability of pellets was assed using granule friabilator (EGF-1, Electrolab India Pvt. Ltd., India). The crushing strength was evaluated by using texture analyzer with P20 type probe (TA-XT *plus*, Stable Micro Systems Ltd., UK). In vitro drug release studies were carried out using USP type II dissolution test apparatus with autosampler assembly (TDT-08L, Electrolab India Pvt. Ltd., India). Residual moisture present in the pellet was analyzed using IR moisture balance (EM-120HR, Adair Dutt & Co. Pvt. Ltd., India). Stability chamber (WIL-195, Wadegati Lab equip Ltd., India) and Humidity Camber (Thermolab Scientific Equipments Pvt. Ltd., India) were used to maintain ambient (25 °C ± 2 °C/60 ± 5% RH) and accelerated (40 °C ± 2 °C/75 ± 5% RH) condition for stability studies. Frost free refrigerator (RT42K5468SL, Samsung Group, South Korea) was used for refrigerated storage.

5.2.3. Preparation of pellets

All ingredients were weighed and sifted through sieve of 425 µm aperture (#40) and dry-blended for 10 min at speed setting 1 of Kenwood mixer. The dry powder blend mass for each batch of pellets was 100 gm. Water was gradually added into the mixture at speed setting 3 of Kenwood mixer and mixing was continued for 6 minutes. The wet mixing process was stopped thrice to scrape the material adhering to the wall of the bowl and the mixing blade. The wet mass was extruded using a single screw extruder at 60 rpm speed. The wet extrudates were immediately transferred to the spheronizer to convert them into pellets. Whenever required, 0.5 to 1% w/w talc was added to spheronizer (at 1000 rpm for 1 min) prior the collection of pellets. Then resulting pellets were collected and dried in single layers in the tray dryer at 60 °C for 4 hr. Finally, dried pellets were screened and suitable size pellets were selected for the further evaluation.

5.2.4. Strategy for formulation development and optimization

Preliminary studies were carried out by preparing dummy pellets (pellets without drug) using Carbopol 971 or Carbopol 974 with Avicel PH101. During these dummy pellets preparations, effect of various process parameters on pellets quality was explored. Subsequently, select process parameters were screened using fractional factorial design (resolution III). Based on the screening study results, critical process parameters were identified which further used for optimization studies.

In case of domperidone, CR pellet formulations were prepared using Carbopol 971, DCP, and Avicel PH101. Release profile was optimized to achieve desired release profile by varying Carbopol 971 and DCP amount. A response surface design i.e. Box-Behnken design was used to investigate the effect of critical process parameters on the quality of pellets and to optimize of developed pellet formulation. In addition, effect of various drying techniques viz. tray drying, air drying and fluidized bed drying on the release profile of optimized pellet formulations was also studied.

In case of venlafaxine hydrochloride, attempts were made to manufacture CR matrix pellets using various levels of Carbopol polymers alone and in combination with other release retarding polymers. Also, Carbopol based CR matrix-membrane pellets were explored. In addition, high drug loading (50 to 70% w/w) were explored using Carbopol 971 as binder. Pellet formulation with 50% w/w venlafaxine hydrochloride was further explored for effect of critical process parameters and optimized using Box-Behnken design.

All experimental design studies, mathematical modeling, validation of models and response surface modeling were carried out using Design Expert® software (version 10.0.7, Stat-Ease Inc., Minneapolis, MN, USA).

5.2.5. Evaluation of the developed pellet formulations

5.2.5.1. Usable yield

Different size fractions of pellets were obtained using electromagnetic sieve shaker which was continuously vibrating at 1 mm amplitude for 20 min [9-10]. The standard set of sieves (595 to 2000 µm) were used for size distribution. For domperidone pellets, fraction of the pellets passed from sieve of 1700 µm aperture (#12) and retained on sieve of 1400 µm aperture (#14) was considered as usable yield. Whereas, for venlafaxine hydrochloride pellets, pellets passed from sieve of 1180 µm aperture (#16) and retained on sieve on sieve of 850 µm aperture (#20) was considered as usable yield [11-12].

5.2.5.2. Pellet size and size distribution analysis

Sieve analysis was carried out for determining the size and size distribution of the pellets. Pellets were separated according to size using sieve shaker as described in section 5.2.5.1. Based on the results, weight distribution was calculated. The pellet size and size distribution was analyzed by calculating the geometric mean diameter and span respectively. The obtained data was processed using log-probability plot according to

Martin [13]. The geometric mean diameter on weight basis (d_g) was calculated from log-probability plot. The d_g is considered as a particle size equivalent to 50% on log-probability scale. Further, span of pellet size distribution was calculated (equation 5.1) by dividing difference obtained between pellets diameter at 90% (D_{90}) and at 10% (D_{10}) by the pellet diameter at 50% (D_{50}) [14-15].

$$\text{Span} = \frac{D_{90} - D_{10}}{D_{50}} \tag{5.1}$$

5.2.5.3. Shape analysis

The modal class fractions, the class wherein maximum fraction of pellets was retained during the sieve analysis, was considered for the shape analysis. A sample of 50 pellets was collected and spread them on wax coated glass slides. Images of the pellets were captured using Nikon D5300 camera. Captured images were processed and shape factors such as roundness and elongation of the pellet images calculated using ImageJ (version 1.51-j8, National Institute of Health, USA) image analysis software. Roundness and elongation were calculated [9,16] using equation 5.2 and 5.3 respectively.

$$\text{Roundness} = \frac{\text{Area}}{\pi \times (\text{Max. radius})^2} \tag{5.2}$$

$$\text{Elongation} = \frac{\text{Max. radius}}{\text{Min. radius}} \tag{5.3}$$

The shape factor, roundness measures the sphericity or circularity of pellets, elongation measures the oblongated shape of pellet.

5.2.5.4. Flow properties

Flowability (flow properties) of designed pellets were evaluated in terms of Hausner's ratio, Carr's index and flow rate [17-18].

Hausner's ratio and Carr's index, the indicators of the flowability, were calculated from bulk and tapped densities of pellets. Pellets were filled in to graduated cylinder up to the 50-ml mark. The weight of pellets required was calculated. The cylinder was then tapped using tapped density apparatus till there was no further change in the volume (as per USP). Bulk density (ρb) was considered as ratio of weight of pellets filled in cylinder to the volume of cylinder occupied by pellets before tapping (i.e. 50 ml). Tapped density (ρt) was considered as ratio of weight of pellets filled in cylinder to the volume of cylinder occupied by pellets after tapping. All the estimations were carried out in

triplicate and Hausner's ratio and Carr's index were calculated as per the equation 5.4 and 5.5 respectively.

$$\text{Hausner's ratio} = \frac{\rho t}{\rho b} \tag{5.4}$$

$$\text{Carr's index} = \frac{\rho t - \rho b}{\rho t} \times 100 \tag{5.5}$$

Further, flow rate of designed pellets was considered as time taken (measured in seconds) by pellets (10 g) to flow through the funnel having internal diameter of 5 mm. All estimations were carried out in triplicates.

5.2.5.5. Friability

The mechanical strength of pellets was estimated by using oscillating apparatus (granule friability tester) as described in European Pharmacopeia (Ph. Eur.). Fine particles on pellets were remove by sieving with sieve of aperture size of 355 µm (#45). In the glass container of volume 105 ml, about 10.00 g (M_1) of the pellets were weighed and container was installed in the apparatus. The container was oscillated for 4 min at 140 oscillations/min. After the test resulted fine particles on pellets were removed using sieving with sieve of aperture size of 355 µm (#45) and determined the weight (M_2). All estimations were carried out in triplicate. The friability of the pellets was calculated as the percentage weight loss was calculated using equation 5.6.

$$\text{Weight loss (\%)} = \frac{M_1 - M_2}{M_1} \times 100 \tag{5.6}$$

Where, M_1: Weight of pellets before test in grams (10 g) and M_2: Weight of pellets after test in grams.

5.2.5.6. Crushing strength

The crushing strength, an estimation of hardness of pellet, was evaluated with the help of texture analyzer [19-20]. P20 type probe was used for crushing of the pellets. 25 pellets from each batch were analyzed by lowering the probe towards individual pellet at 0.1 mm s^{-1}. The force required to crush the pellet (in Newton) was measured when the probe goes below to 50% of the height of the pellet. The crushing strength of pellet was represented as the arithmetic mean of the force required for 25 pellets.

5.2.5.7. Residual moisture content

Infrared (IR) moisture balance was used for analyzing residual moisture present in the pellet after drying [21]. The equipment was pre-calibrated and standardized with starch. 2 gm of pellets were placed on IR moisture balance and temperature was set at 105°C. The time point at which two successive readings matches each other considered as endpoint. Each pellets batch was analyzed in triplicate.

5.2.5.8. Estimation of drug content

The pellets equivalent to 1 gm were weighed accurately and crushed using mortar and pestle. From the resulting powder, a 10 mg of sample was weighed and transferred to 100 ml volumetric flask. The volume was made up with phosphate buffer (25 mM, pH 3.0) and further sonicated for 10 min for complete extraction of drug. After sonication samples were filtered through 0.22 µm Millipore® syringe filters (Millex® Syringe Filter, Millipore). The resulting samples diluted and analyzed using HPLC methods as described in Chapter 3.

5.2.5.9. In vitro drug release studies

In vitro drug release studies for designed pellets were carried out using USP type II dissolution test apparatus. For domperidone pellets, drug release study was carried out in 900 ml 0.1 N HCl maintained at 37.0 ± 0.5 °C. The paddle rotation speed was set at 100 rpm. The pellets equivalent to 30 mg of domperidone pellets were charged in dissolution medium. At predetermined time intervals, samples were withdrawn and analyzed by using UV spectroscopic method as discussed in Chapter 3 (Part I-A).

For venlafaxine hydrochloride pellets, drug release study was carried out in 900 distilled water maintained at 37.0 ± 0.5 °C. The paddle rotation speed was kept at 100 rpm. The pellets equivalent to 37.5 mg of venlafaxine hydrochloride were charged in dissolution medium. At predetermined time intervals, samples were withdrawn and analyzed by using UV spectroscopic method as discussed in Chapter 3 (Part II-A).

a. Model independent approach

In order to facilitate the interpretation and comparison of the dissolution profiles of designed formulations, model-independent approaches such as mean dissolution time (MDT), dissimilarity (f_1) and similarity (f_2) factors were used.

MDT is the arithmetic mean of dissolution profile [22-23] was calculated using equation 5.7:

$$MDT = \frac{\sum_{j=1}^{n} t_{mid}\ \Delta M}{\sum_{j=1}^{n} \Delta M} \quad (5.7)$$

Where, j is the dissolution sample number, n is the number of sampling time points, t_{mid} is the time at the midpoint between t_j and t_{j-1} and ΔM is the amount of drug dissolved between t_j and t_{j-1}.

f_1 and f_2 factors were used to statistically compare the dissolution pattern of the samples [24-25]. f_1 is defined as the measure of percent error between two dissolution profiles. f_2 is a logarithmic transformation of the sum-squared error of the difference between the percentage dissolved of test and reference product over all time points. Furthermore, f_2 value was calculated for assessing the similarity of dissolution profiles as recommended in the FDA Guidance for Industry [26-27]. These values were calculated using equation 5.8 and 5.9:

$$f_1 = \left\{ \frac{[\sum_{t=1}^{n} |R_t - T_t|]}{\sum_{t=1}^{n} R_t} \right\} \times 100 \quad (5.8)$$

$$f_2 = 50 \log \left\{ \left[1 + (1/n) \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \quad (5.9)$$

Where n is number of sampling points, R_t and T_t are the cumulative drug release from reference and test sample at each sampling point t, respectively.

The f_1 factor assumes value of 0 in case of identical dissolution profiles of the test and reference. The value of f_1 increases with increase in the dissimilarity. Generally, f_1 value within range of 0 to 15 indicates similarity in dissolution profile. The f_2 value of 100 indicates the identical dissolution profile of the test and reference. Higher f_2 value, in the range of 50 and 100 indicates similar dissolution profile and values of f_2 lesser than 50 signify dissimilarity. The dissolution data used for comparison was obtained under the same test conditions and their dissolution time points were the same.

b. Model dependent approach

The kinetics of drug release from the various dosage form can be understood by comparing drug release data either with theoretically proven release kinetic mechanisms such as zero order, first order or with empirical equations (Hixson-Crowell, Weibull, etc.) suggested by several researchers for variety of formulations [28-31]. Therefore, obtained in vitro dissolution study data of CR pellets was fitted to different mathematical

models to assess the drug release kinetics (zero order and first order model) and release mechanism (Hixson-Crowell model, Weibull model, Higuchi model, Korsemeyer-Peppas model, Hopfenberg model, and Baker-Lonsdale model).

5.2.5.10. Batch reproducibility

To study batch reproducibility, batches of optimized formulations of both drugs were manufactured in triplicates keeping similar processing conditions. Triplicate samples from each batch were evaluated for all quality parameters discussed earlier.

5.2.5.11. Stability studies of formulations

To evaluate the stability, batches of pellet formulations were packed in hermetically sealed vials and stored at different storage conditions such as long term stability condition or controlled room temperature (CRT: 25 ± 2 °C / 60 ± 5% RH), accelerated condition (AT: 40 ± 2 °C / 75 ± 5% RH) and refrigerated condition (FT: 5 ± 2 °C) as per ICH and WHO stability testing guidelines [32,33]. At predetermined time intervals (0, 1, 3 and 6 months for AT condition; 0, 1, 3, 6, 9 and 12 months for CRT condition and FT condition), samples were withdrawn and evaluated for physical observations, drug content and in vitro release studies. The drug content in the samples was analyzed using validated stability indicating HPLC methods (Chapter 3). All samples were analyzed in triplicate. The results of quality control tests of stability samples were compared with initial (zero-time) results. The percentage drug remaining to be degraded (%RTD) was plotted against time in order to calculate degradation rate constant K_{deg} and $t_{90\%}$ value at different storage conditions for all the formulations.

5.3. Result and Discussion

5.3.1. Formulation development and optimization

5.3.1.1. Preliminary studies

As described earlier, two Carbopol polymers viz. Carbopol 971 and Carbopol 974 were selected for the present research work. Initially, dummy pellets (pellets without drug) were manufactured using Carbopol 971 (5%, 10%, 15%, 20 w/w) and Carbopol 974 (5%, 10%, 15%, 20%, 25% w/w) with Avicel PH101. In case of Carbopol 971, dummy pellets having acceptable shape and yield were obtained up to 10% w/w level. However, with 15% w/w of Carbopol 971, only dumbbells were observed and use of 20% w/w Carbopol 971, resulted in very sticky extrudates which formed big lumps during spheronization. Similarly, in case of Carbopol 974, dummy pellets having acceptable shape and yield

were observed up to 15% w/w level. Above that level dumbbells or sticky big lumps were observed.

Further, various grades of microcrystalline cellulose (MCC) viz. Avicel PH101, Avicel PH102, Cyclocel 101, Cyclocel 102 and low particle size (about 5-10 µm) MCC were explored for dummy pellet preparation. It was observed that Avicel PH 101 has highest water holding capacity during wet mixing and it produced pellets with better yield and shape. In a separate study, dummy pellets were prepared by addition Carbopol 974 dispersed in water. It was compared with the pellet formulation where Carbopol 974 was added in dry powder form. The formulation with Carbopol dispersion resulted in very sticky extrudates and pellets. Moreover, aggregates of pellets were obtained after drying. Based on these observations, for further studies, Avicel PH101 was selected as spheronization aid and Carbopol was added in dry powder form.

5.3.1.2. Screening of process variables with fractional factorial design

Mostly, fractional factorial design with resolution III and IV are used for screening studies. Although resolution III design requires very few experimental runs and can estimate main interactions, it can be confounded by two factor interactions. On the other hand, resolution IV design requires comparatively more number of experimental runs, but it can estimate main interaction as well as two factor interactions. Resolution IV designs can be confounded by three factor interactions, but these higher-order interactions are less likely to be significant compared to low-order interactions. So, a resolution IV design is superior than a resolution III design as it has less-severe confounding pattern. Designs with resolution V and higher are rarely used for screening as number of experimental runs are high which it is not economical and sensible [34-35]. Thus, fraction factorial design with resolution IV was selected screening study.

For the present research work, planetary mixer was used for both dry and wet mixing. Thus, reaming two process variables involved in mixing viz. amount of granulation fluid and wet mixing time were selected for screening. As present research requires dense pellets, dome extruder with single screw was used for the pellet preparation [36-37] and pellet size was decided based on the final formulation requirement. Consequently, only remaining process variable associated with extrusion process i.e. extrusion speed was included in the screening studies. Cross-hatch patterned friction plate was selected for this study as it is most preferred friction plate for the spheronization process [38-39]. So,

remaining all process variables involved in spheronization process viz. spheronization load, spheronization speed and spheronization time were selected for the screening studies. Drying technique mainly influence release properties of pellets. A separate study was carried out to explore the effect of various drying techniques (viz. tray drying, air drying and fluidized bed drying) on release profile. Hence, process variables associated with drying process were excluded from the screening studies.

Based on the above selected variables, a six factor, two level, fractional factorial design (Resolution IV) was used as a screening design to identify the significant formulation and process variables that affect pellet shape and yield. Selected process variables (independent variables) and their levels are summarized in Table 5.1. Yield and pellet shape were selected as dependent variables. Experimental runs generated for this design and their observed response for 16 pellet formulations are summarized in Table 5.2.

Half normal plots generated by Design-Expert® software for the effect of process parameters on yield and pellet shape are shown in Figure 5.1 and 5.2 respectively. These plots are primary tools for selection of process parameters which have impact on the selected response. The terms which fall below or right side of the red line likely to have repeatable and significant effect. Figure 5.1 and 5.2 indicates that yield and pellet shape are likely to have significant impact of three process parameters viz. water level, spheronization speed and spheronization time. Shapiro-Wilk p-value was found to be greater than 0.10 for yield (p-value: 0.814) and pellet shape (p-value: 0.615) indicating that all terms with significant effect were selected in half normal plot. Similar observations were made using pareto charts (Figure 5.3 and 5.4). In pareto charts, effects that are above the Bonferroni limit are almost certainly important and while effects that are above the t-value limit are possibly important. So, Figure 5.3 and 5.4 also indicates that only three process parameters viz. water level, spheronization speed and spheronization time may have significant effect on yield and pellet shape. Using this information, important terms were selected, and ANOVA studies were carried out.

Results of ANOVA studies for yield and shape are presented in Table 5.3 and 5.4 respectively. Table 5.3 indicates that factorial model for the effect of process parameters on yield was found to be significant as P value is < 0.0001.

Table 5.1. Fractional factorial design (FFD): selected process variables and their level

Level	Water (%)	Wet mixing time (min)	Extrusion speed (rpm)	Spheronization speed (rpm)	Spheronization time (min)	Spheronization load (gm)
Low (-1)	40	4	50	1400	3	50
High (1)	80	8	100	2800	9	100

Table 5.2. Fractional factorial design (FFD): experimental runs and effect of independent process variables on dependent variables

Batch code	Run	Water (%)	Wet mixing time (min)	Extrusion speed (rpm)	Spheronization speed (rpm)	Spheronization time (min)	Spheronization load (gm)	Shape	Yield (%)
DP/FFD/01	1	80	8	50	2800	3	50	0.8771	78.11
DP/FFD/02	2	80	8	100	2800	9	100	0.9012	72.23
DP/FFD/03	3	40	4	50	2800	3	100	0.7416	53.76
DP/FFD/04	4	80	4	50	1400	9	50	0.8015	86.12
DP/FFD/05	5	40	4	50	1400	3	50	0.6552	75.89
DP/FFD/06	6	40	4	100	2800	9	50	0.7605	41.76
DP/FFD/07	7	80	4	100	2800	3	50	0.8653	78.54
DP/FFD/08	8	80	4	100	1400	3	100	0.7616	91.45
DP/FFD/09	9	80	8	50	1400	3	100	0.7580	90.23
DP/FFD/10	10	80	4	50	2800	9	100	0.9052	71.54
DP/FFD/11	11	40	8	100	1400	3	50	0.6432	70.36
DP/FFD/12	12	40	8	50	1400	9	100	0.6753	71.15
DP/FFD/13	13	80	8	100	1400	9	50	0.8104	84.20
DP/FFD/14	14	40	8	100	2800	3	100	0.7398	54.65
DP/FFD/15	15	40	8	50	2800	9	50	0.7118	42.61
DP/FFD/16	16	40	4	100	1400	9	100	0.6687	70.43

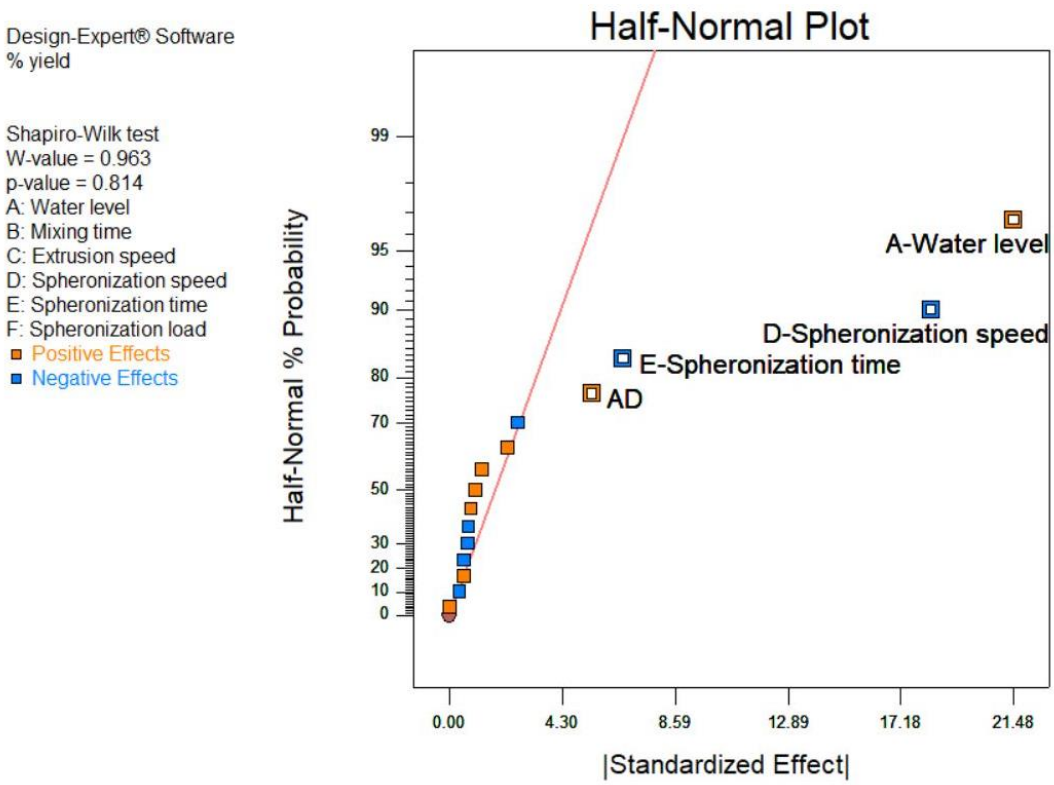

Figure 5.1. Fractional factorial design: half normal plot for effect of process parameters on % yield

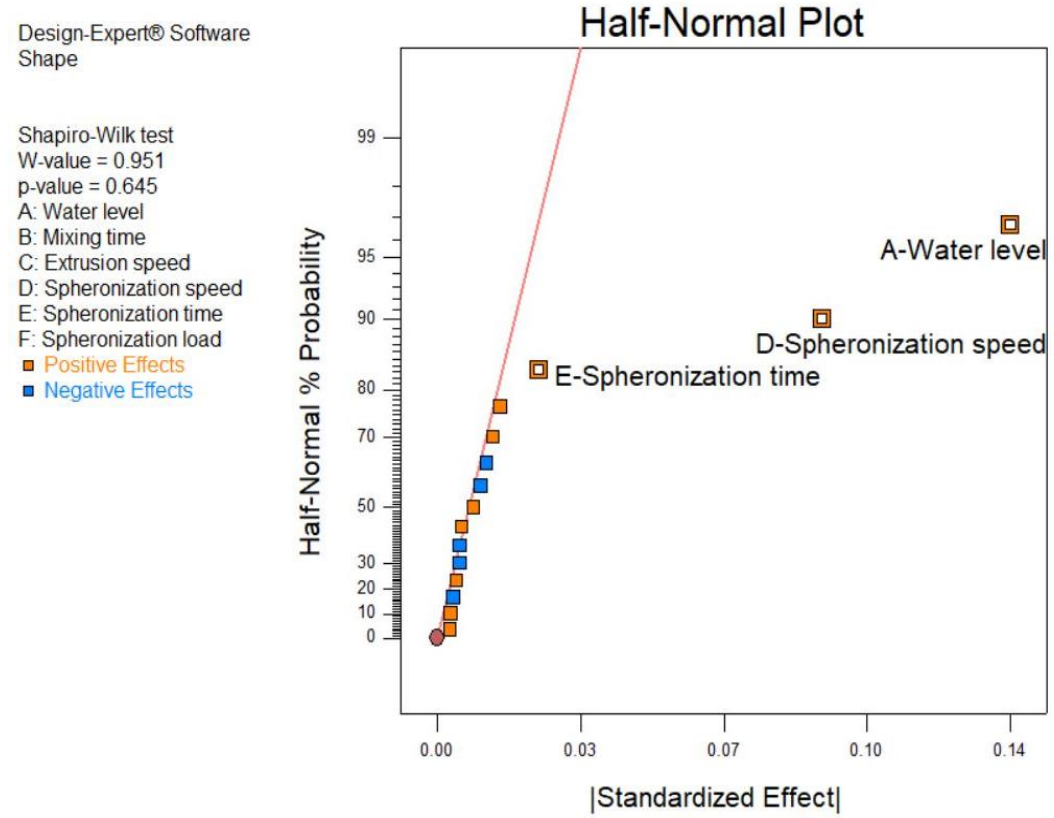

Figure 5.2. Fractional factorial design: half normal plot for effect of process parameters on shape

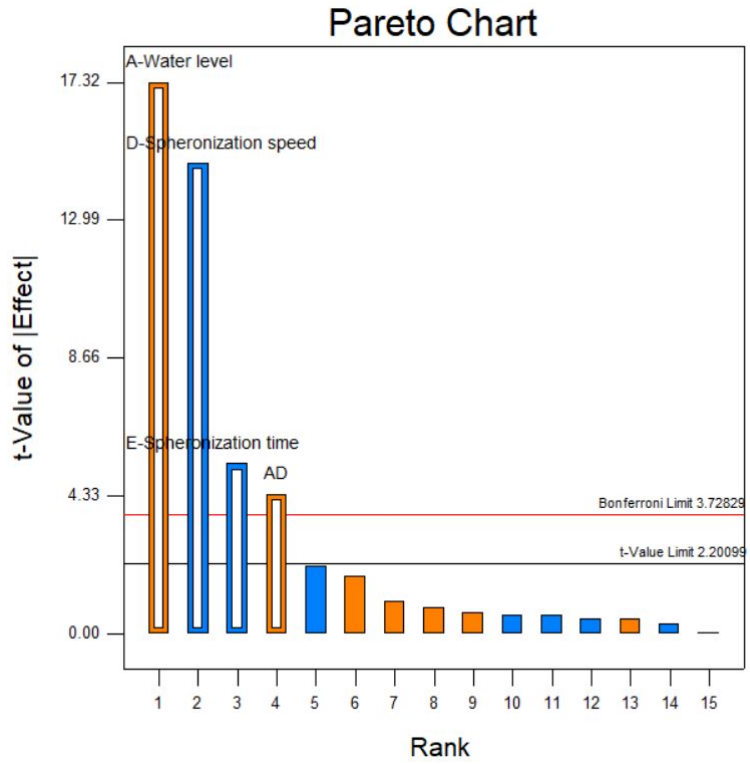

Figure 5.3. Fractional factorial design: Pareto chart for effect of process parameters on % yield

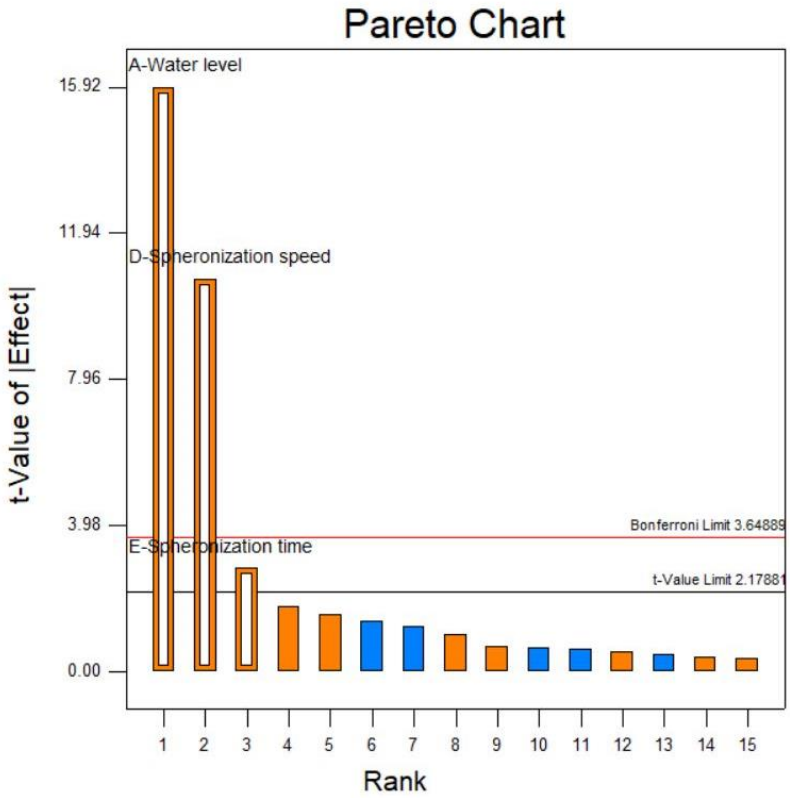

Figure 5.4. Fractional factorial design: Pareto chart for effect of process parameters on shape

The predicted R-squared value (0.9613) was in reasonable agreement with the adjusted R-squared value (0.9450) i.e. the difference was less than 0.2. P value less than 0.05 for water level, spheronization speed and spheronization time indicates that these parameters have significant effect on yield. Also, two factor interaction effect of water level and spheronization time (AD) was observed on pellet yield.

Table 5.3. Fractional factorial design: ANOVA for selected factorial model (for yield)

Source	Sum of squares	df	Mean square	F value	P-value (Prob > F)	
Model	3482.02	4	870.51	141.52	< 0.0001	significant
A-Water level	1844.92	1	1844.92	299.93	< 0.0001	
D-Spheronization speed	1343.77	1	1343.77	218.46	< 0.0001	
E-Spheronization time	175.23	1	175.23	28.49	0.0002	
AD	118.10	1	118.10	19.20	0.0011	
Residual	67.66	11	6.15			
Cor Total	3549.69	15				

Similarly, Table 5.4 indicates that factorial model for the effect of process parameters on shape was also found to be significant (p-value: < 0.0001). The predicted R-squared value (0.9740) was in reasonable agreement with the adjusted R-squared (0.9597). Pellet shape also significantly influenced by only three process parameters viz. water level, spheronization speed and spheronization time (p-values: < 0.05).

Table 5.4. Fractional factorial design: ANOVA for selected factorial model (for shape)

Source	Sum of squares	df	Mean square	F value	P-value (Prob > F)	
Model	0.11	3	0.036	125.31	< 0.0001	significant
A-Water level	0.073	1	0.073	253.46	< 0.0001	
D-Spheronization speed	0.033	1	0.033	114.46	< 0.0001	
E-Spheronization time	2.323E-003	1	2.323E-003	8.02	0.0151	
Residual	3.478E-003	12	2.899E-004			
Cor Total	0.11	15				

In summary, it can be concluded that out of six selected process parameters, only three process parameters viz water level, spheronization speed and spheronization time have significant effect on yield and pellet shape. Thus, these three parameters were identified as critical process parameters and further used for the optimization studies.

5.3.1.3. Development of domperidone pellets

5.3.1.3.1. Preliminary studies for development of CR domperidone pellets

Preliminary studies for domperidone pellet formulation studies were carried out to match target release profile set by Lubrizol which is summarized in Table 5.5. Selected formulation batches explored during preliminary studies and their release profiles are summarized in Table 5.6.

Table 5.5. Target release profile for domperidone controlled release pellets

Time point (hr)	% in vitro release
1	15 to 40%
2	30 to 60%
4	55 to 85%
12	Not less than 70%

During initial trials, mainly dumbbells were observed during spheronization. The probable reason being higher elasticity of extrudates which can be attributed to rheological properties of Carbopol 971. Thus, to decrease elasticity and increase brittleness, a high-density material i.e. DCP was added in formulation. Also, it was observed that DCP along with Carbopol polymers showed slightly enhanced release retardation. Furthermore, DCP is much more economical than Avicel PH101. Thus, to improve pellets properties and to make formulation cost effective, DCP was used in all domperidone CR formulations.

Formulation batches from DOM/PS/01 to DOM/PS/04 were formulated with 40% domperidone. These formulation trial batches prepared with Carbopol 971 (10-15% w/w) and Carbopol 974 (15-20% w/w). Only, formulation batch with 15% w/w of Carbopol 974 (DOM/PS/03) yielded pellets however it failed achieve desired release profile. So, in attempt achieve desired release profile, batches from DOM/PS/05 to DOM/PS/12 were formulated with 30% w/w domperidone. Formulation prepared with 5, 7.5 and 10% w/w Carbopol 971 (DOM/PS/05 to DOM/PS/07) yielded pellets but failed to achieve target release profile. However, all batches showed better release retardation than DOM/PS/03.

Table 5.6. Preliminary study batches of domperidone controlled release pellets

Batch code	Drug (%)	Carbopol 971 (%)	Carbopol 974 (%)	Avicel PH101 (%)	DCP (%)	Dissolution data (N = 6) (% release ± SD)				Observations
						1 hr	2 hr	4 hr	12 hr	
DOM/PS/01	40	10	-	40	10	-	-	-	-	Extrudates breaking at very short length and only fines were formed during spheronization
DOM/PS/02	40	15	-	35	10	-	-	-	-	Very sticky extrudates and only big lumps were observed during spheronization
DOM/PS/03	40	-	15	35	10	46.48 ± 0.45	72.65 ± 0.85	88.68 ± 0.66	93.86 ± 0.78	Pellets with wider size distribution, low yield but good sphericity
DOM/PS/04	40	-	20	30	10	-	-	-	-	Very sticky extrudates and only big lumps were observed during spheronization
DOM/PS/05	30	5	-	55	10	44.43 ± 0.65	69.10 ± 0.99	81.58 ± 0.59	87.67 ± 1.15	Pellets with wider size distribution and low sphericity
DOM/PS/06	30	7.5	-	52.5	10	42.62 ± 0.65	67.43 ± 0.87	77.11 ± 0.15	84.40 ± 0.59	Pellets with wider size distribution and low sphericity
DOM/PS/07	30	10	-	50	10	41.23 ± 0.39	65.10 ± 0.30	74.12 ± 1.22	82.45 ± 1.10	Pellets with wider size distribution and low sphericity
DOM/PS/08	30	15	-	45	10	-	-	-	-	Very sticky extrudates and only big lumps were observed during spheronization
DOM/PS/09	30	12.5	-	47.5	10	-	-	-	-	Very sticky extrudates and only big lumps were observed during spheronization
DOM/PS/10	30	-	15	45	10	42.18 ± 0.23	72.11 ± 1.39	84.58 ± 0.41	91.04 ± 1.65	Pellets with better size distribution and shape than DOM/PS/01 to DOM/PS/07 batches

Table 5.6. Preliminary study batches of domperidone controlled release pellets (continued)

Batch code	Drug (%)	Carbopol 971 (%)	Carbopol 974 (%)	Avicel PH101 (%)	DCP (%)	Dissolution data (N = 6) (% release ± SD)				Observations
						1 hr	2 hr	4 hr	12 hr	
DOM/PS/11	30	-	20	40	10	42.46 ± 0.19	69.65 ± 0.50	85.86 ± 0.70	90.86 ± 0.52	Over-wetted pellets and lumps were observed during spheronization
DOM/PS/12	30	-	25	35	10	-	-	-	-	Very sticky extrudates and only big lumps were observed during spheronization
DOM/PS/13	20	5	-	65	10	44.06 ± 0.54	68.64 ± 0.67	81.61 ± 0.85	89.68 ± 0.56	Pellets with wider size distribution and low sphericity
DOM/PS/14	20	7.5	-	72.5	-	36.33 ± 1.18	64.94 ± 0.34	78.16 ± 0.23	87.34 ± 0.45	Dumb-bells and pellets with wider size distribution
DOM/PS/15	20	7.5	-	62.5	10	34.23 ± 0.24	61.23 ± 0.40	74.78 ± 0.31	83.50 ± 0.54	Pellets with wider size distribution and low sphericity
DOM/PS/16	**20**	**7.5**	-	**52.5**	**20**	**32.22 ± 0.45**	**58.29 ± 0.55**	**73.47 ± 0.46**	**81.50 ± 0.31**	Pellets with wider size distribution and better sphericity than batch DOM/PS/16
DOM/PS/17	**20**	**7.5**	-	**47.5**	**25**	**30.55 ± 0.69**	**55.72 ± 0.26**	**71.11 ± 0.40**	**80.33 ± 0.38**	Pellets with wider size distribution, lot of fines, vey low yield
DOM/PS/18	20	10	-	45	25	-	-	-	-	Wider pellet size distribution and poor yield compared to batch DOM/PS/18
DOM/PS/19	**20**	**9**	-	**49**	**22**	**27.29 ± 0.71**	**52.29 ± 0.53**	**68.83 ± 0.43**	**76.14 ± 0.56**	Pellets with lower yield and better sphericity

Formulation trials with Carbopol 971 level of 12.5% and higher (DOM/PS/08 and DOM/PS/09) were produced sticky wet extrudates and big lumps during spheronization. Similarly, pellets formulations with 15 to 25% w/w Carbopol 974 (DOM/PS/10 and DOM/PS/12) were attempted, however these batches showed poor release retardation compared to formulation batches with Carbopol 971. Consequently, domperidone level was further reduced to achieve desired release profile.

Formulation batches form DOM/PS/13 to DOM/PS/19 were prepared with 20% w/w domperidone and Carbopol 971 (5-10% w/w). Formulation batch with 7.5% w/w level (DOM/PS/16) of Carbopol 971 showed release profile within desired limits. Further, to study the effect of DCP on drug release profile, DOM/PS/14 to DOM/PS/17 batches were prepared with 0-25% w/w of DCP level. It was observed that increased DCP level showed slightly enhanced release retardation (Table 5.6). Further, pellet batch with 10% w/w Carbopol 971 and 25% w/w DCP (DOM/PS/18) was also attempted. However, frequent blocking of extrusion screen as well as temperature rise during extrusion, and overwetted pellets were observed in formulation trials with 25% w/w DCP. Therefore, formulation batch with 9% w/w Carbopol 971 and 22% w/w DCP (DOM/PS/19) was attempted. This batch showed best release retardation and no blocking was observed during extrusion. However, batch yield was less 60% and roundness value of 0.598. Therefore, there was need to be improve yield as well as pellets shape. Thus, this batch was further optimized using response surface design.

5.3.1.3.2. Optimization study for domperidone CR pellet formulation

Box-Behnken design is useful for the investigation of quadratic response surfaces. Also, it generates a second order polynomial model using a small number of experimental runs which helps in optimization studies [40-41]. Thus, for the optimization of domperidone controlled release pellet formulation, Box-Behnken design was employed. Three process variables viz. water level, spheronization speed and spheronization time were identified as critical process parameters during screening studies. These three parameters selected as independent variables and were investigated at three different levels (Table 5.7). Yield and pellet shape were selected as dependent variables. Experimental runs generated for this design and their observed response for 17 pellets formulation batches are summarized in Table 5.8.

Table 5.7. Box-Behnken design (BBD) for domperidone pellets: selected process variables and their level

Level	Water (%)	Spheronization speed (rpm)	Spheronization time (min)
Low (-1)	50	1200	3
Medium (0)	70	2000	6
High (1)	90	2800	9

Table 5.8. Box-Behnken design (BBD) for domperidone pellets: experimental runs and effect of independent process variables on dependent variables

Batch code	Run	Water (%)	Sph. speed (rpm)	Sph. time (min)	Yield (%)	Shape
DOM/BBD/01	1	90	2000	9	64.85	0.9274
DOM/BBD/02	2	70	1200	3	74.10	0.8456
DOM/BBD/03	3	50	2000	9	44.37	0.7192
DOM/BBD/04	4	90	2000	3	70.45	0.9117
DOM/BBD/05	5	70	2800	9	74.57	0.9256
DOM/BBD/06	6	70	2000	6	87.15	0.9105
DOM/BBD/07	7	50	1200	6	59.67	0.6855
DOM/BBD/08	8	70	2000	6	87.15	0.9105
DOM/BBD/09	9	70	1200	9	75.57	0.8656
DOM/BBD/10	10	70	2000	6	87.15	0.9105
DOM/BBD/11	11	50	2800	6	34.85	0.7716
DOM/BBD/12	12	70	2000	6	87.15	0.9105
DOM/BBD/13	13	90	2800	6	60.78	0.9376
DOM/BBD/14	14	90	1200	6	65.78	0.8995
DOM/BBD/15	15	50	2000	3	52.17	0.7018
DOM/BBD/16	16	70	2000	6	87.15	0.9105
DOM/BBD/17	17	70	2800	3	81.45	0.9014

5.3.1.3.2A. Optimization steps for Box-Behnken design

Design-Expert® software was used for all mathematical calculations. Initially, regression calculations to fit all of the polynomial models to the selected responses were carried out. It generated statistics such as p-values, lack of fit, Predicted Residual Sum of Squares (PRESS) values and R-squared values for the comparison of all models. Polynomial model with highest R-squared value and lowest PRESS value was selected for the generation of polynomial equation. Also, there should be reasonable agreement between Adjusted R-squared and Predicted R-squared values (within 0.2 of each other) for the selected model. Selected model was further validated using ANOVA. Then, polynomial equations involving the main response and interaction factors was generated based on various parameters like predicted residual sum of squares, multiple correlation

coefficients and adjusted multiple correlation coefficient. Additionally, the validation of these generated polynomial equations was carried out statistically using ANOVA. Finally, 3D response surface plots were obtained to study the effects of the independent factors on the responses viz. yield and particle shape.

5.3.1.3.2B. Effect of critical process parameters (independent factors) on yield:

All regression and statistics values generated for all polynomial model is summarized in Table 5.9. Quadratic polynomial model was selected for further analysis based on R-squared and PRESS values comparison as discussed in section 5.3.1.3.2A. Summary of ANOVA study performed carried out for the validation of selected model is presented in Table 5.10. Model was found to be significant as P value < 0.0001. The Predicted R-Squared value (0.8863) was in reasonable agreement with the Adjusted R-Squared (0.9918) i.e. the difference was less than 0.2. Adequate precision (measures signal to noise ratio) was found to be 47.59 (greater than 4 is desirable) which indicates an adequate signal. So, this model can be used to navigate the design space.

The polynomial equation generated for quadratic model for yield was:

$$\% \text{ yield} = 87.15000 + 8.85000 \times A + 1.58750 \times B - 2.35125 \times C + 4.95500 \times AB - 2.08750 \times BC - 25.17125 \times A^2 - 6.70875 \times B^2 - 4.01875 \times C^2 - 9.04250 \times A^2B \quad (5.10)$$

where, A is water level (%), B is spheronization speed (rpm) and C is spheronization time (min)

3D response surface plot generated for yield is shown in Figure 5.5. As indicated in Figure 5.5A and 5.5B, initially as water level was increased, pellet yield was found to be increased and then after certain limit, increase in water level caused decrease in yield. This behavior can be explained as follows. Very low water level must have caused rough extrudates which may result in wider size distribution. Also, there was inadequate moisture during spheronization, which lead to lot of dust formation. Due to this, initially yield was low very low. As water level increased, extrudates become smoother and sufficient moisture content during spheronization helped to densify the pellets and reduced fine which resulted in increased yield. However, after certain level, increased water level caused overwetted mass with agglomeration of the individual pellets in to larger lumps during spheronization which resulted in reduced yield [7]. Figure 5.5A shows that, at lower water level, significant decrease in yield was observed with higher spheronization speed. The reason behind this is the increased amount of fine.

Table 5.9. Box-Behnken design (BBD) for domperidone pellets: model summary statistic for yield

Source	Standard deviation	R-squared	Adjusted R-squared	Predicted R-squared	PRESS	
Linear	16.12	0.1797	-0.0096	-0.3827	5691.23	
2FI	18.05	0.2081	-0.2670	-1.6788	11025.51	
Quadratic	**5.03**	**0.9570**	**0.9016**	**0.7113**	**2834.53**	**Suggested**
Cubic	0.000	1.0000	1.0000		+	Aliased

Table 5.10. Box-Behnken design (BBD) for domperidone pellets: ANOVA for response surface model (for yield)

Source	Sum of squares	df	Mean square	F value	P- Value (Prob > F)	
Model	4101.06	9	455.67	215.02	< 0.0001	significant
A-Water level	626.58	1	626.58	295.66	< 0.0001	
B-Spheronization speed	10.08	1	10.08	4.76	0.0655	
C-Spheronization time	44.23	1	44.23	20.87	0.0026	
AB	98.21	1	98.21	46.34	0.0003	
BC	17.43	1	17.43	8.22	0.0241	
A^2	2667.76	1	2667.76	1258.82	< 0.0001	
B^2	189.50	1	189.50	89.42	< 0.0001	
C^2	68.00	1	68.00	32.09	0.0008	
A^2B	163.53	1	163.53	77.17	< 0.0001	
Residual	14.83	7	2.12			
Lack of Fit	14.83	3	4.94			
Pure Error	0.000	4	0.000			
Cor Total	4115.90	16				

Increased spheronization speed generates more frictional force which causes increased amount of fine especially with underwet granulation. On the contrary, Figure 5.5C indicates that, at moderate water level, spheronization speed has resulted in slight increase in yield. At optimum moisture level, increase in spheronization speed results in increased densification and decreased amount of fines which explains slights increase in yield [7,17]. Yield was also affected by spheronization time. The negative coefficient of C in equation 5.10 suggested a negative relation between spheronization time and pellet yield.

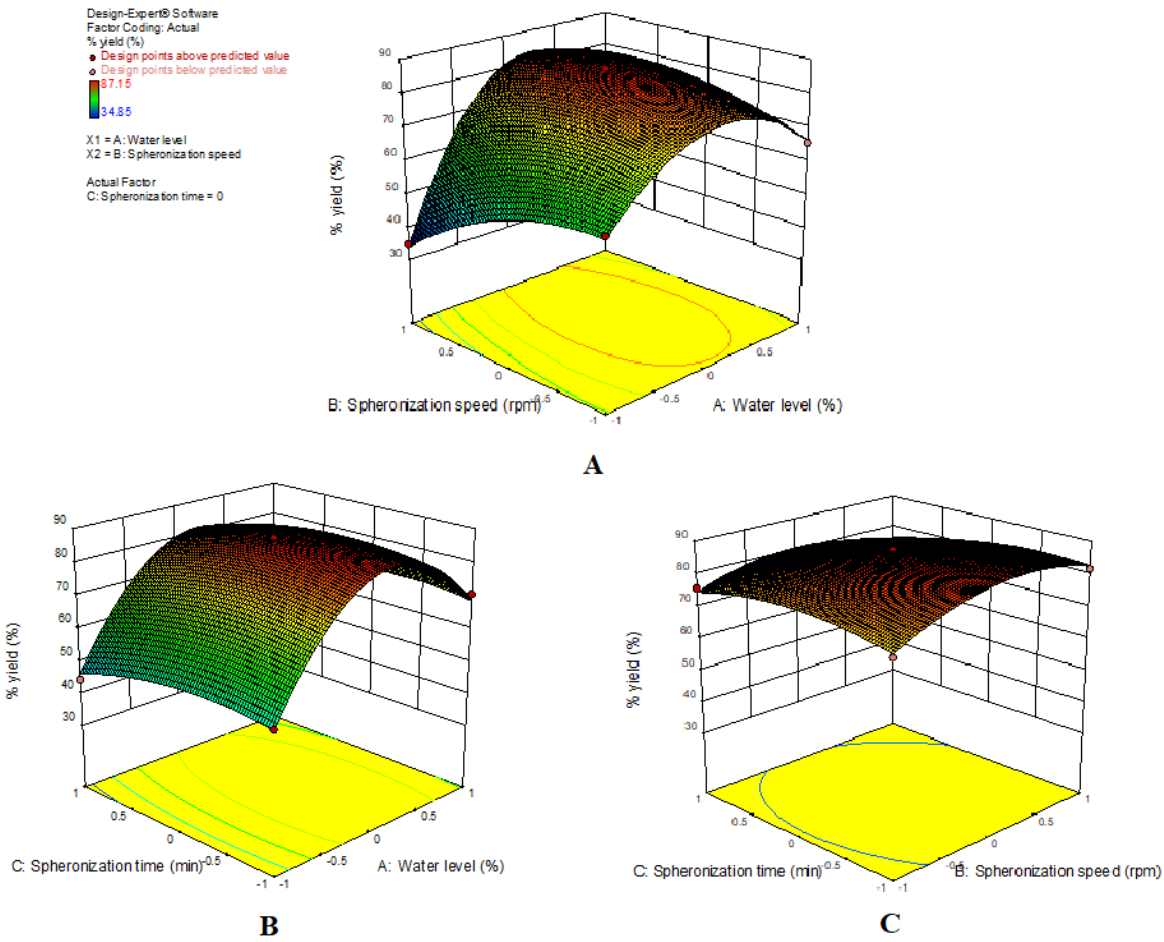

Figure 5.5. Box-Behnken design (BBD) for domperidone pellets: Response surface for yield A. effect of spheronization speed (rpm) and water level (%); B. effect of spheronization time (min) and water level (%); C. effect of spheronization time (min) and spheronization speed (rpm)

Figure 5.5C indicates, spheronization time has very little effect on yield at lower spheronization speed however longer spheronization time significantly reduced yield at higher spheronization speed. It also evident by negative coefficient of BC term in equation 5.10. The higher speed and longer spheronization time increased the moisture loss during the process. This loss moisture resulted in large amount of fines which explains the lower yield [42].

5.3.1.3.2C. Effect of critical process parameters (independent factors) on yield:

Similarly, all regression and statistics values generated for all polynomial model for effect of process parameters on pellet shape is summarized in Table 5.11. Quadratic polynomial model was selected for further analysis based on R-squared and PRESS values comparison as discussed in section 5.3.1.3.2A. Summary of ANOVA study performed carried out for the validation of selected model is presented in Table 5.12.

Model was found to be significant as P value < 0.0001. The Predicted R-Squared value (0.9718) was in reasonable agreement with the Adjusted R-Squared (0.9960) i.e. the difference was less than 0.2. Adequate precision (measures signal to noise ratio) was found to be 62.42 (greater than 4 is desirable) which indicates an adequate signal. So, this model can be used to navigate the design space.

Table 5.11. Box-Behnken design (BBD) for domperidone pellets: model summary statistic for pellet shape

Source	Standard deviation	R-squared	Adjusted R-squared	Predicted R-squared	PRESS	
Linear	0.047	0.7492	0.6913	0.5741	0.050	
2FI	0.054	0.7541	0.6066	0.1491	0.099	
Quadratic	**0.0054**	**0.9982**	**0.9960**	**0.9718**	**0.00329**	**Suggested**
Cubic	0.000	1.0000	1.0000		+	Aliased

Table 5.12. Box-Behnken design (BBD) for domperidone pellets: ANOVA for response surface model (for pellet shape)

Source	Sum of squares	df	Mean square	F value	P- Value (Prob > F)	
Model	0.12	9	0.013	441.24	< 0.0001	significant
A-Water level	0.080	1	0.080	2709.86	< 0.0001	
B-Spheronization speed	7.200E-003	1	7.200E-003	245.05	< 0.0001	
C-Spheronization time	7.469E-004	1	7.469E-004	25.42	0.0015	
AB	5.760E-004	1	5.760E-004	19.60	0.0031	
AC	7.225E-007	1	7.225E-007	0.025	0.8798	
BC	4.410E-006	1	4.410E-006	0.15	0.7100	
A^2	0.026	1	0.026	877.18	< 0.0001	
B^2	3.196E-004	1	3.196E-004	10.88	0.0132	
C^2	1.251E-003	1	1.251E-003	42.58	0.0003	
Residual	2.057E-004	7	2.938E-005			
Lack of Fit	2.057E-004	3	6.856E-005			
Pure Error	0.000	4	0.000			
Cor Total	0.12	16				

The polynomial equation generated for quadratic model for yield was:

$$\text{Shape} = 0.91050 + 0.099763 \times A + 0.030000 \times B - 0.00966 \times C - 0.01200 \times AB - 0.000425 \times AC + 0.00105 \times BC - 0.078238 \times A^2 - 0.00871 \times B^2 - 0.017237 \times C^2 \quad (5.11)$$

where, A is water level (%), B is spheronization speed (rpm) and C is spheronization time (min).

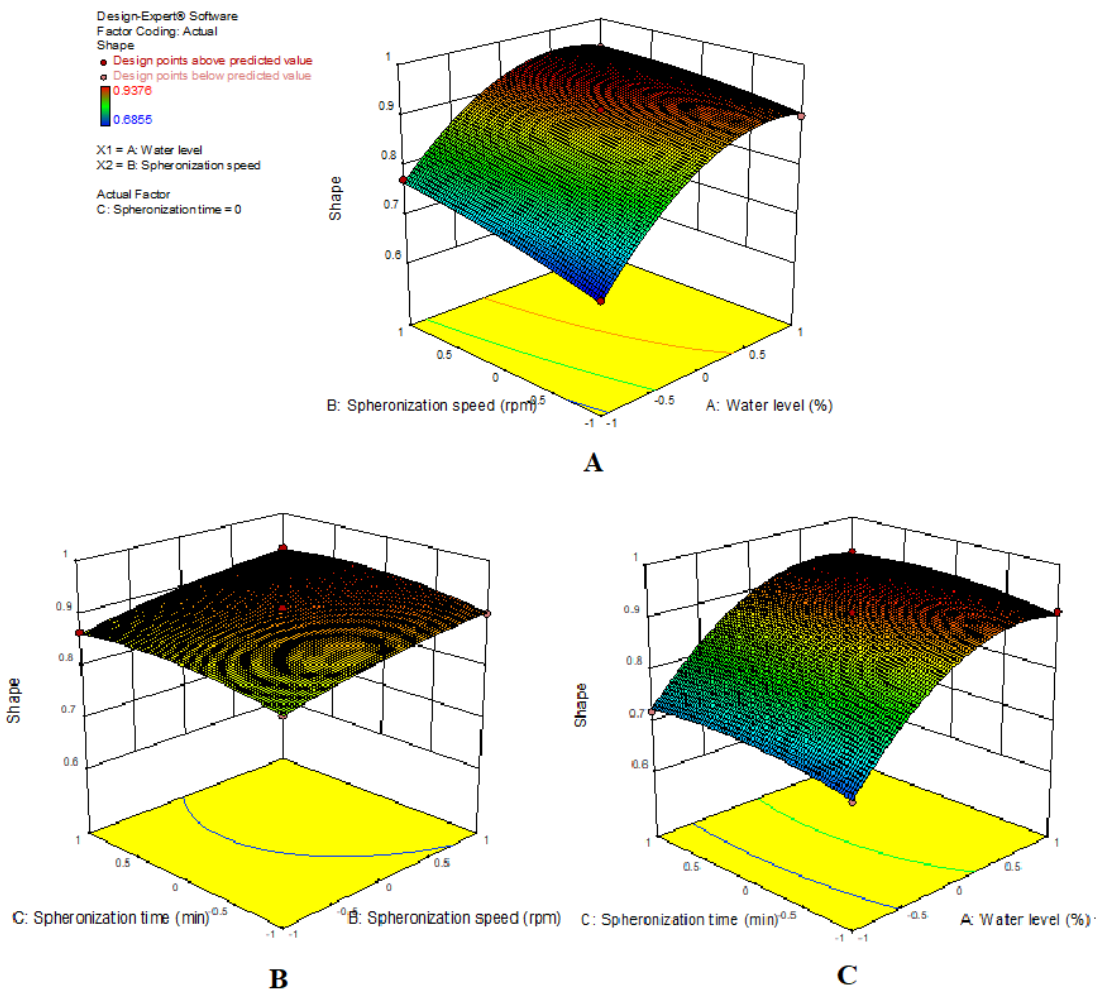

Figure 5.6. Box-Behnken design (BBD) for domperidone pellets: Response surface for shape A. effct of spheronization speed (rpm) and water level (%); B. effect of spheronization time (min) and spheronization speed (rpm); C. effect of spheronization time (min) and water level (%)

3D response surface plot generated for pellet shape is shown in Figure 5.6. Large coefficient of term A in equation 5.11 as well as Figure 5.6A and 5.6C, indicates that water level has large effect on pellet shape. The reason behind that is, lower moisture level during spheronization decreases densification of pellets which results in pellet with rough surface and inadequate sphericity. Figure 5.6A shows that higher spheronization speed increase the sphericity of pellets. This is an expected behavior as higher speed increase the overall frictional and centrifugal force which results into densification and improved sphericity of pellets [6,43]. Figure 5.6B, indicates that pellets with highest sphericity was obtained at optimum water level with faster spheronization speed and longer spheronization time. However as indicated in Figure 5.6C, at lower water level, spheronization time does not have significant effect on pellet shape.

5.3.1.3.2D. Optimization and validation

The Design-Expert® software was used to predict the optimized conditions for optimized formulations. The criteria set for optimized formulations were minimum spheronization time, roundness factor above 0.85 and maximum yield. Predicted factor levels were transformed in actual values using following equation 5.12 [44].

$$\text{Coded factor level} = \frac{\text{X - the average of the two levels}}{\text{one-half the difference of two levels}} \quad (5.12)$$

Where X is actual value of factor.

New pellet formulations batches of domperidone with levels of factors predicted by software were prepared to analyze the validity of the selected optimization models and generated equations. The predicted factor levels (coded) and their actual factor value after transformation are summarized in Table 5.13. Predicted response values as well as practically observed response values are summarized in Table 5.14.

Table 5.13. Box-Behnken design (BBD) for domperidone pellets: predicted factor levels (coded) and their actual factor value after transformation

Batch code	Coded factor level			Actual factor values		
	Water level	Sph. speed	Sph. time	Water level (%)	Sph. speed (rpm)	Sph. time (min)
DOM/BBD/OB/1	0.279	0.104	-0.452	75.58	2083.2	4.644
DOM/BBD/OB/2	0.038	0.074	-0.386	70.76	2059.2	4.842
DOM/BBD/OB/3	0	0	0	70.00	2000.0	6.000
DOM/BBD/OB/4	0.280	0.165	-0.709	75.60	2132.0	3.873
DOM/BBD/OB/5	0.088	0.488	-0.022	71.76	2390.4	5.934

Table 5.14. Box-Behnken design (BBD) for domperidone pellets: predicted response values as well as practically observed response values

Batch code	Predicted response		Actual response	
	Yield (%)	Shape	Yield (%)	Shape
DOM/BBD/OB/1	88.16	0.9270	89.56	0.9356
DOM/BBD/OB/2	87.91	0.9100	86.51	0.9067
DOM/BBD/OB/3	87.15	0.9105	88.60	0.9120
DOM/BBD/OB/4	87.73	0.9209	89.54	0.9167
DOM/BBD/OB/5	87.16	0.9305	85.98	0.9123

So, pellet formulations with better shape and yield were with shorter processing time were successfully prepared with using this optimized method (Table 5.14). These optimized batches were used for further studies.

5.3.1.3.3. Effect of drying techniques on the release profile of domperidone CR pellets

Many researchers have studied and documented the effect of drying techniques on mechanical properties and drug release behavior of pellets [45-47]. Thus, to study the effect of drying technique on drug release, formulation batches similar in composition to DOM/BBD/OB/1 were prepared and dried using three different techniques viz. tray drying (DOM/BBD/OB/1), fluidized bed drying (DOM/BBD/OB/1FBD) and air drying (DOM/BBD/OB/1AD). Tray drying was performed at 60 ℃ for 4 hr while air drying was carried out at room temperature for 24 hr. Parameters set for fluidized bed drying were as follows: inlet temperature: 50 to 55 ℃; product temperature: 38 to 42 ℃; outlet temperature: 34 to 36 ℃; and blower speed: 60 rpm.

Release profiles of these batches are shown in Figure 5.7. Further, these release profiles were compared with each other using f_1 and f_2 factors. In all the cases f_2 value was found to be more than 90 while f_1 value was found to be lower than 15. These results indicate that all release profiles are similar and these drying technique does not influence drug release behavior of Carbopol based CR formulations of domperidone. These results indicate that, to reduce overall processing time, faster drying technique such fluidized bed dryer can be used instead of tray dryer without affecting drug release performance of Carbopol based pellet formulations.

5.3.1.3.4. In vitro release performance comparison between Carbopol based matrix CR pellets and coated CR pellets

Furthermore, release profile of this final optimized batch of Carbopol based matrix CR pellet formulation of domperidone prepared using extrusion-spheronization (DOM/BBD/OB/1) was compared with coated CR pellet formulation of domperidone prepared using dry powder layering and fluidized bed processor (DOM/PL-FBP/1). Release profiles for both these formulations is shown in Figure 5.8. The f_1 and f_2 value were found to be 3.94 and 73.945 respectively which indicates that release profiles are similar for both these formulations. So, these results demonstrate that Carbopol based CR matrix pellets can be developed using extrusion-spheronization technology and these developed matrix pellet formulations can exhibit similar release profile to that of coated CR pellet formulations.

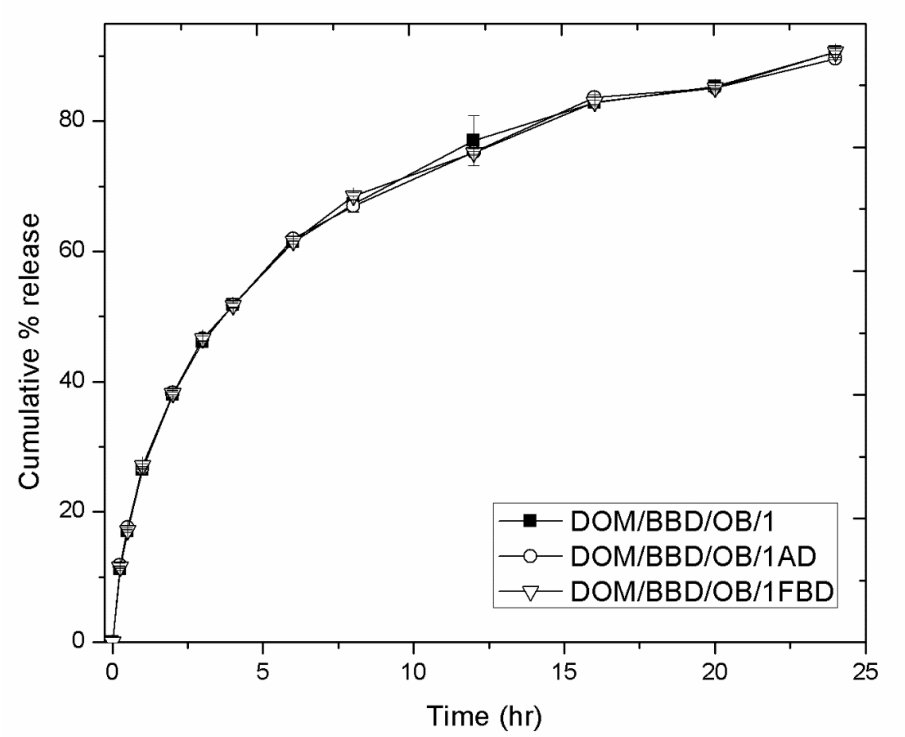

Figure 5.7. Effect of drying techniques on in vitro drug release profile of domperidone CR pellet formulation

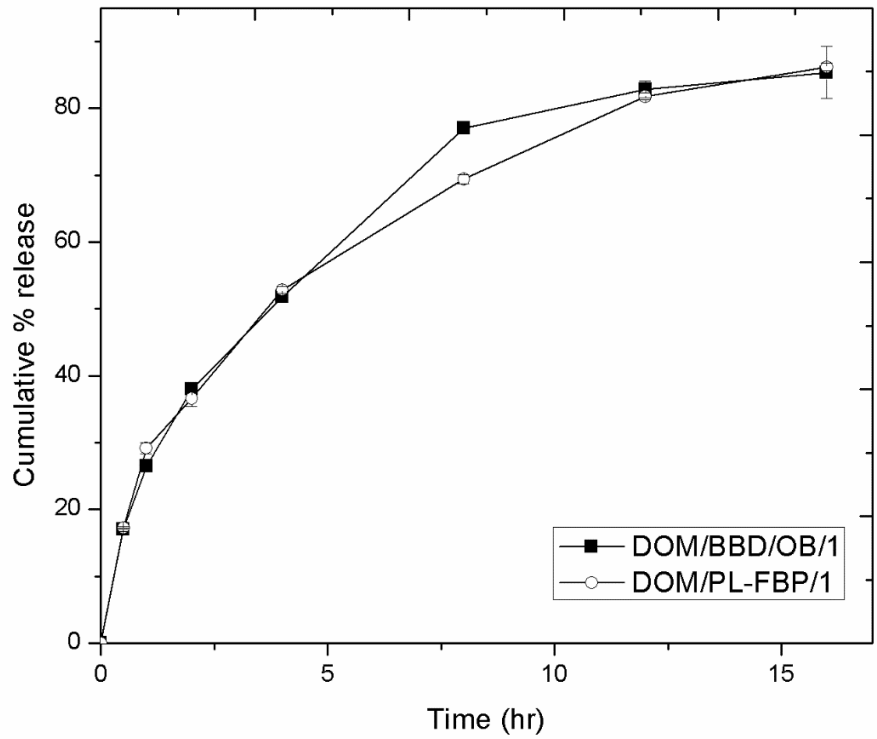

Figure 5.8. In vitro drug release profile for DOM/BBD/OB/1 (Carbopol based CR matrix pellets of domperidone) and DOM/PL-FBP/1 (Coated CR pellets of domperidone)

5.3.1.4. Development of venlafaxine hydrochloride pellets

5.3.1.4.1. Development of CR pellets of venlafaxine hydrochloride

Initially, in an attempt to develop CR formulation, pellets loaded with 30% w/w venlafaxine and various levels of Carbopol 971 (5%, 7.5%, 10%, 15%, 20% and 30% w/v) were formulated. Pellets with reasonable quality (yield ≥ 50%; roundness ≥ 0.6) were obtained with up to 20% w/w of Carbopol 971. However, these formulations failed to prolong the release of the drug and more than 90% of the drug was released within 1 hr during in vitro release studies. So, amount of venlafaxine was reduced in subsequent trials.

Pellets loaded with 20% w/w venlafaxine with Carbopol 971 (10%, 15%, 20%, 25% and 30% w/w) as well as Carbopol 974 (15%, 20%, 25% and 30% w/w) were attempted. In addition, use of non-aqueous granulation fluid such as isopropyl alcohol and ethyl acetate were explored. Further, combination of other release retarding agents such as xanthan gum, hydroxy propyl cellulose, etc. along with 10-20% w/w of Carbopol 971 or 20% w/w of Carbopol 974 were attempted. Some of the trials yielded pellets with reasonable quality (yield ≥ 50%; roundness ≥ 0.6) however release retardation was not improved significantly, and most efficient formulation trial also showed more than 70% of drug release within 1 hr.

Finally, preparation of CR pellets with matrix-membrane technology were attempted. In this, core pellets were prepared using Carbopol 971 with extrusion-spheronization technology. Further, 20 to 30% w/w of Carbopol 971 along with DCP was coated on the pellets using dry powder layering method with the help of a pan coater. Yet again, no formulation with significant prolonged release was obtained.

5.3.1.4.2. Development of high drug loaded IR pellets of venlafaxine hydrochloride

As described in section 5.3.1.4.1., Carbopol polymers are unable to control the release of highly soluble drugs in pellet formulations. Nevertheless, Carbopol polymers can also be used as a binder. Thus, it was decided to explore applicability of Carbopol polymers in immediate release (IR) pellets for highly soluble drugs. These pellets can be directly used as IR formulation or can be used as core pellets for further coating using fluidized bed processor or pan coater, etc. to develop modified release formulations. Currently, core IR pellets are manufactured with powder layering method. Compared to drug layering method, Carbopol based IR pellets using extrusion-spheronization produces denser, smoother and more spherical pellets which are more suitable for coating purpose. Besides, it does require very less processing time. Although, polyvinyl pyrrolidine (PVP) is commonly used as a binder in extrusion-spheronization, it requires additional mixing

step to prepare an aqueous solution to use it as binder. On the other hand, addition of low amount of Carbopol powder in mixing step can provide excellent binding. Considering these possible advantages, high drug loaded IR pellets of venlafaxine hydrochloride were developed and optimized. Key formulation batches prepared for venlafaxine hydrochloride during preliminary studies were summarized in Table 5.15.

Table 5.15. Preliminary study batches of venlafaxine hydrochloride IR pellets

Batch code	Drug (%)	Carbopol 974 (%)	Avicel PH101 (%)	Water level (%)	Observations
VEN/PS/01	50	10	40	40	Very sticky wet mass, extrusion screen was blocked
VEN/PS/02	50	7.5	42.5	40	Very sticky wet mass, extrusion screen was blocked
VEN/PS/03	50	5	45	40	Very sticky wet mass, extrusion screen was blocked
VEN/PS/04	50	2.5	47.5	40	Very sticky wet mass, extrusion screen was blocked
VEN/PS/05	50	2.5	47.5	30	Very sticky wet mass, extrusion screen was blocked
VEN/PS/06	**50**	**2.5**	**47.5**	**20**	Good smooth extrudates, mixture of pellets and dumbbells were observed
VEN/PS/07	60	2.5	37.5	20	Slightly sticky extrudates, overwetted pellet aggregates
VEN/PS/08	**60**	**2.5**	**37.5**	**17.5**	Good extrudates and pellets with acceptable shape were observed after spheronization
VEN/PS/09	70	2.5	27.5	20	Very sticky wet mass and extrudates, extrusion screen was blocked
VEN/PS/10	70	2.5	27.5	17.5	Very sticky wet mass and extrudates, extrusion screen was blocked
VEN/PS/11	70	2.5	27.5	15	Good extrudates but blocking extrusion screen, only fine were produced after spheronization

As indicated in Table 5.15, IR pellets of venlafaxine hydrochloride were formulated with 50 to 70% w/w of drug loading. Formulation bathes from VEN/PS/01 to VEN/PS/06

were prepared with 50% w/w venlafaxine hydrochloride and varied amount of Carbopol 974 (2.5-20% w/w). Formulation batch VEN/PS/06 yielded pellets with reasonable quality (yield: 51.56; roundness: 0.674). During the preparation of formulation batch VEN/PS/06, wet mass and extrudates were slightly sticky. Although, no blockage of extrusion screen was observed, pellets were sticky and adhered to the spheronizer wall during spheronization. In addition, aggregates of pellets were observed after drying. So, 1% w/w talc was added to spheronizer (at 1000 rpm and spheronized for 1 min) prior the collection of pellets. Addition of talc prevented sticking pellets to the spheronizer wall and also prevented formation of aggregates of pellets after drying. Thus, further all batches, 1% w/w talc was added at the end of spheronization step.

Further formulation batch VEN/PS/08 revealed that pellets reasonable quality (yield: 68.37; roundness: 0.635) can be prepared with 60% w/w venlafaxine hydrochloride. However, to improve shape, size distribution and achieve better yield, extrusion screen with higher length to diameter (L/R) ratio need to be explored. Pellets with 70% w/w drug loading (VEN/PS/09 to VEN/PS/011) were also tried but yielded poor quality pellets (yield ≤ 15%) with frequent blocking of extruder. Different type extruder with higher L/R ratio need to be explored which may yield better quality pellets with 70% w/w venlafaxine. Based on these observations, formulation batch VEN/PS/06 was selected for further optimization studies to improve pellets shape and yield.

5.3.1.4.3. Optimization study for venlafaxine hydrochloride IR pellet formulation

Box-Behnken Design was selected for optimization study as it allows optimization study with lowest number of runs. Similar to the optimization study of domperidone CR pellets, three process variables viz. water level, spheronization speed and spheronization time at three level were employed for optimization study (Table 5.16). Yield and pellet shape were selected as dependent variables. Experimental runs generated with this design and their observed response for 17 formulation batches of venlafaxine IR pellets are summarized in Table 5.17.

Table 5.16. Box-Behnken design (BBD) for venlafaxine hydrochloride pellets: selected process variables and their level

Level	Water (%)	Spheronization speed (rpm)	Spheronization time (min)
Low (-1)	20	1200	2
Medium (0)	23	2000	5
High (1)	26	2800	8

Table 5.17. Box-Behnken design (BBD) for venlafaxine hydrochloride pellets: experimental runs and effect of independent process variables on dependent variables

Batch code	Run	Water (%)	Sph. speed (rpm)	Sph. time (min)	Yield (%)	Shape
VEN/BBD/01	1	23	2800	2	90.55	0.7423
VEN/BBD/02	2	23	1200	2	71.34	0.6317
VEN/BBD/03	3	23	2000	5	89.55	0.8812
VEN/BBD/04	4	23	2000	5	89.55	0.8812
VEN/BBD/05	5	23	2000	5	89.55	0.8812
VEN/BBD/06	6	20	2800	5	75.12	0.7745
VEN/BBD/07	7	23	2000	5	89.55	0.8812
VEN/BBD/08	8	20	2000	2	63.24	0.6221
VEN/BBD/09	9	26	2000	8	69.13	0.9267
VEN/BBD/10	10	26	2000	2	70.12	0.7615
VEN/BBD/11	11	23	1200	8	76.21	0.7117
VEN/BBD/12	12	23	2800	8	83.89	0.9342
VEN/BBD/13	13	23	2000	5	89.55	0.8812
VEN/BBD/14	14	20	2000	8	74.88	0.7292
VEN/BBD/15	15	20	1200	5	60.55	0.5415
VEN/BBD/16	16	26	2800	5	74.12	0.9285
VEN/BBD/17	17	26	1200	5	61.56	0.6812

5.3.1.4.3A. Effect of critical process parameters (independent factors) on yield:

All regression and statistics values generated for all polynomial model is summarized in Table 5.18. Quadratic polynomial model was selected for further analysis as it has highest R-squared value and lowest PRESS values (as discussed in section 5.3.1.3.2A).

Table 5.18. Box-Behnken design (BBD) for venlafaxine hydrochloride pellets: model summary statistic for yield

Source	Standard deviation	R-squared	Adjusted R-squared	Predicted R-squared	PRESS	
Linear	10.72	0.2004	0.0159	-0.3028	2436.18	
2FI	11.92	0.2400	-0.2159	-1.3625	4417.72	
Quadratic	**1.67**	**0.9896**	**0.9762**	**0.8331**	**312.13**	**Suggested**
Cubic	0.000	1.0000	1.0000		+	Aliased

Summary of ANOVA study performed carried out for the validation of selected model is presented in Table 5.19. Model was found to be significant as P value < 0.0001. The Predicted R-Squared value (0.8331) was in reasonable agreement with the Adjusted R-

Squared (0.9762) as the difference was less than 0.2. Adequate precision (measures signal to noise ratio) was found to be 22.73 (greater than 4 is desirable) which indicates an adequate signal. So, this model can be used to navigate the design space.

Table 5.19. Box-Behnken design (BBD) for venlafaxine hydrochloride pellets: ANOVA for response surface model (for yield)

Source	Sum of squares	df	Mean square	F value	P- Value (Prob > F)	
Model	1850.40	9	205.60	73.77	< 0.0001	significant
A-Water level	0.16	1	0.16	0.058	0.8161	
B-Spheronization speed	364.77	1	364.77	130.89	< 0.0001	
C-Spheronization time	9.81	1	9.81	3.52	0.1027	
AB	1.01	1	1.01	0.36	0.5661	
AC	39.88	1	39.88	14.31	0.0069	
BC	33.24	1	33.24	11.93	0.0106	
A^2	1137.13	1	1137.13	408.03	< 0.0001	
B^2	117.33	1	117.33	42.10	0.0003	
C^2	59.96	1	59.96	21.52	0.0024	
Residual	19.51	7	2.79			
Lack of Fit	19.51	3	6.50			
Pure Error	0.000	4	0.000			
Cor Total	1869.91	16				

The polynomial equation generated for selected quadratic model for the prediction of yield was:

$$\% \text{ yield} = 89.55 + 0.14 \times A + 6.75 \times B + 1.11 \times C - 0.50 \times AB - 3.16 \times AC - 2.88 \times BC - 16.43 \times A^2 - 5.28 \times B^2 - 3.77 \times C^2 \quad (5.13)$$

where, A is water level (%), B is spheronization speed (rpm) and C is spheronization time (min).

3D response surface plot generated for yield is shown in Figure 5.9. Figure 5.9A and 5.9B and large coefficients of term A and B indicates that both water level and spheronization speed has large effect on yield. As indicated in Figure 5.9A, lower water level resulted in lower yield. This can be explained by formation of fines due to low moisture level during spheronization. Also Figure 5.9A, shows that, higher level of water also resulted in lower yield. At higher water level, overwetted mass during wet mixing and sticky extrudates were observed which formed lumps during spheronization. This lump formation resulted in lower yield.

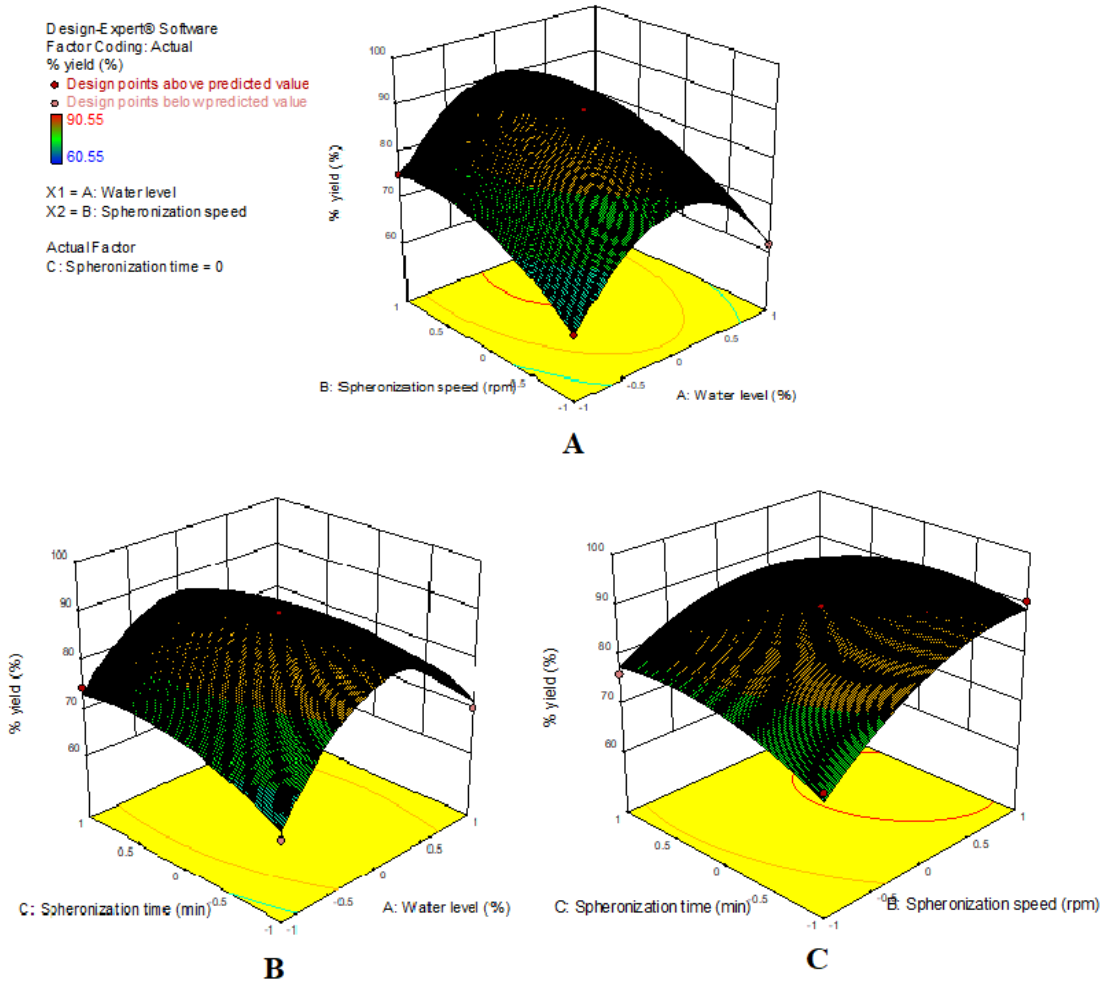

Figure 5.9. Box-Behnken design (BBD) for venlafaxine IR pellets: Response surface for yield A. effect of spheronization speed (rpm) and water level (%); B. effect of spheronization time (min) and water level (%); C. effect of spheronization time (min) and spheronization speed (rpm)

Figure 5.9B shows that longer spheronization time resulted in slightly decreased yield. This effect was more significant at lower water level. This effect is also evident with larger coefficient of term AC in equation in 5.13. The probable reason behind that can be a moisture loss during longer spheronization time which resulted in generation of fine especially, in formulation trials with lower water level. Figure 5.9C shows that longer spheronization time with higher speed resulted in decreased yield. Higher spheronization speed intensifies frictional force which may results in fine generation with longer spheronization time.

5.3.1.4.3B. Effect of critical process parameters (independent factors) on shape:

Similarly, all regression and statistics values generated for all polynomial model for effect of process parameters on pellet shape is summarized in Table 5.20. Model with

highest R-squared value and lowest PRESS values i.e. quadratic polynomial model was selected.

Table 5.20. Box-Behnken design (BBD) for venlafaxine hydrochloride pellets: model summary statistic for pellet shape

Source	Standard deviation	R-squared	Adjusted R-squared	Predicted R-squared	PRESS	
Linear	0.074	0.7055	0.6375	0.5590	0.11	
2FI	0.082	0.7223	0.5556	0.3025	0.17	
Quadratic	**0.021**	**0.9878**	**0.9720**	**0.8040**	**0.047**	**Suggested**
Cubic	0.000	1.0000	1.0000		+	Aliased

Summary of ANOVA study performed carried out for the validation of selected model is presented in Table 5.21. Model was found to be significant as P value < 0.0001. The Predicted R-Squared value (0.8040) was in reasonable agreement with the Adjusted R-Squared (0.97200) i.e. the difference was less than 0.2. Adequate precision (measures signal to noise ratio) was found to be 25.32 (greater than 4 is desirable) which indicates an adequate signal. So, this model can be used to navigate the design space.

Table 5.21. Box-Behnken design (BBD) for venlafaxine hydrochloride pellets: ANOVA for response surface model (for pellet shape)

Source	Sum of squares	df	Mean square	F value	P- Value (Prob > F)	
Model	0.24	9	0.026	62.72	< 0.0001	significant
A-Water level	0.050	1	0.050	118.28	< 0.0001	
B-Spheronization speed	0.083	1	0.083	196.79	< 0.0001	
C-Spheronization time	0.037	1	0.037	88.09	< 0.0001	
AB	5.112E-005	1	5.112E-005	0.12	0.7375	
AC	8.439E-004	1	8.439E-004	2.01	0.1994	
BC	3.130E-003	1	3.130E-003	7.45	0.0294	
A^2	0.022	1	0.022	52.57	0.0002	
B^2	0.025	1	0.025	59.92	0.0001	
C^2	0.010	1	0.010	23.95	0.0018	
Residual	2.942E-003	7	4.203E-004			
Lack of Fit	2.942E-003	3	9.806E-004			
Pure Error	0.000	4	0.000			
Cor Total	0.24	16				

The polynomial equation generated for quadratic model for pellet shape was:

$$\text{Shape} = 0.8812 + 0.079 \times A + 0.10 \times B + 0.068 \times C + 0.00357 \times AB + 0.015 \times AC + 0.28 \times BC - 0.072 \times A^2 - 0.77 \times B^2 - 0.049 \times C^2 \quad (5.14)$$

where, A is water level (%), B is spheronization speed (rpm) and C is spheronization time (min).

3D response surface plot generated for pellet shape is shown in Figure 5.10. Coefficient of term A, B and C in equation 5.14 indicates that all three process parameters viz. water level, spheronization speed and spheronization time have positive effect. It also shows that spheronization speed has large effect on sphericity. Figure 5.10C showed that higher spheronization speed and with spheronization time resulted in pellets with highest sphericity. This effect is also evident with larger coefficient of term BC in equation in 5.14. As described earlier, larger speed increases the effect of frictional and centrifugal force which leads to smoother and more spherical pellets.

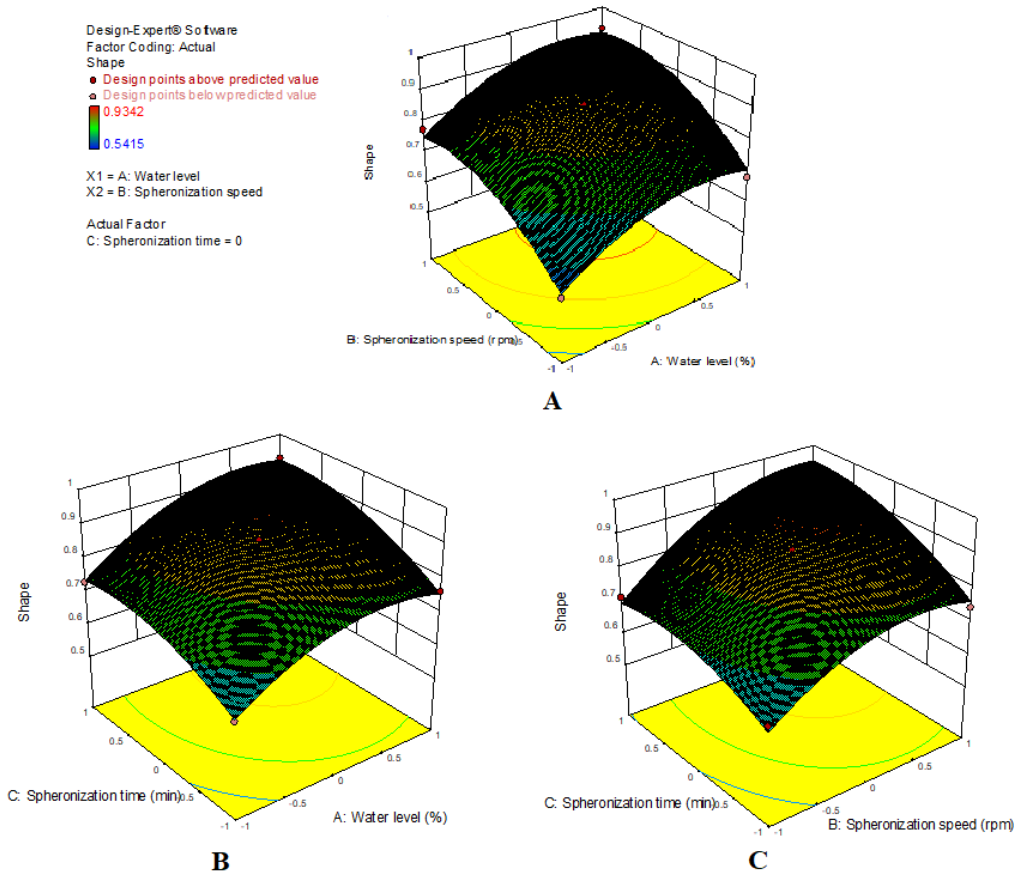

Figure 5.10. Box-Behnken design (BBD) for venlafaxine IR pellets: Response surface for pellet shape A. effect of spheronization speed (rpm) and water level (%); B. effect of spheronization time (min) and water level (%); C. effect of spheronization time (min) and spheronization speed (rpm)

5.3.1.4.3C. Optimization and validation

The Design-Expert® software was used to predict the optimized conditions for optimized formulations. The criteria set for optimized formulations were minimum spheronization time, roundness factor above 0.88 and maximum yield. Predicted factor levels were transformed in actual values using equation 5.12. Using these predicted factor levels, formulations batches of venlafaxine IR pellets were prepared to analyze the validity of the selected optimization models and generated equations. The predicted factor levels (coded) and their actual factor value after transformation are summarized in Table 5.22. Predicted response values as well as practically observed response values are summarized in Table 5.23.

Table 5.22. Box-Behnken design (BBD) for venlafaxine hydrochloride pellets: predicted factor levels (coded) and their actual factor value after transformation

Batch code	Coded factor level			Actual factor values		
	Water level	Sph. speed	Sph. time	Water level (%)	Sph. speed (rpm)	Sph. time (min)
VEN/BBD/OB/1	0.005	0.669	-0.111	23.02	2535	4.56
VEN/BBD/OB/2	-0.043	0.601	-0.219	22.87	2481	4.12
VEN/BBD/OB/3	0.034	0.652	0.098	23.10	2522	5.39
VEN/BBD/OB/4	0.080	0.177	-0.155	23.24	2142	4.38
VEN/BBD/OB/5	-0.108	0.281	-0.002	22.68	2223	4.99

Table 5.23. Box-Behnken design (BBD) for venlafaxine hydrochloride pellets: predicted response values as well as practically observed response values

Batch code	Predicted response		Actual response	
	Yield (%)	Shape	Yield (%)	Shape
VEN/BBD/OB/1	91.74	0.9047	90.12	0.9123
VEN/BBD/OB/2	90.83	0.8939	90.10	0.8967
VEN/BBD/OB/3	91.56	0.9251	90.13	0.9178
VEN/BBD/OB/4	91.60	0.8899	90.70	0.9012
VEN/BBD/OB/5	90.83	0.8939	90.10	0.8967

So, venlafaxine hydrochloride IR pellet formulations with better shape (more than 0.9) and yield (more than 90%) were successfully prepared shorter processing time with using this optimized method (Table 5.23). These optimized batches were used for further studies.

5.3.1.5. Evaluation of pellets

Various pellet evaluation studies were carried out for selected pellet formulation of domperidone and venlafaxine hydrochloride. All these selected batches were prepared in triplicate and evaluated.

5.3.1.5.1. Usable yield

A well optimized pellet formulation using extrusion-spheronization process, should produce a high percentage of pellets within expected size range with acceptable shape as well as sufficient mechanical robustness [11-12]. Optimized batches of domperidone and venlafaxine hydrochloride showed % yield in the range of 89.56 to 90.70% (Table 5.24). The high % yield observed for optimized batches showed that various formulation and process parameters selected for the preparation of domperidone CR matrix pellets and venlafaxine hydrochloride IR pellets using extrusion-spheronization were optimum.

5.3.1.5.2. Pellet size and size distribution analysis

Pellets size and size distribution data for domperidone CR pellets and high drug loaded venlafaxine pellets is summarized in Table 5.24. The geometric mean diameter of the domperidone CR pellets was found to be in the range of 1.515 to 1.559 mm whereas for venlafaxine pellets, it was in the range of 1.007 to 1.019 mm. As pellet size increase, its surface area decreases which results into lower drug release from formulation. Thus, in attempt to achieve target release profile, higher size pellets were developed for domperidone CR formulations. On other hand, lower size pellets were developed for high drug loaded venlafaxine hydrochloride as these batches are intended to be used for further coating process which requires lower size pellet fraction. Span value is measure of width of particle size distribution. Lower value of span indicates narrow pellet size distribution. As indicated in Table 5.24, optimized batches of both domperidone and venlafaxine hydrochloride showed lower span values (0.624 to 0.687) than preliminary study batches (0.827 to 0.868). These span values for final selected pellets formulation batches indicated narrow size distribution. To conclude, lower standard deviation values for pellet size and lower span values confirms that designed pellet formulation process is well optimized.

5.3.1.5.3. Shape analysis

Shape i.e. roundness of pellets is essential evaluation parameter as it can influence flow properties, coating performance and release properties of pellets. Shape of pellet was

assessed in terms of roundness factor and elongation. Results of shape analysis data for domperidone and venlafaxine hydrochloride pellets is summarized in Table 5.24. The roundness value of more than 0.88 was accepted as round pellets [43]. Elongation which is also known as aspect ratio should be closer to 1. Roundness values for optimized formulations were observed in the range of 0.896 to 0.936 which indicates that optimized pellet formulations have acceptable shape.

5.3.1.5.2. Flow properties

The flow properties of pellets were determined by evaluation of bulk density, tapped density, Carr's index, Hausner's ratio and flow rate. Both, domperidone CR pellets and venlafaxine pellets showed excellent flow properties. Results of all these flow properties studies for both domperidone and venlafaxine pellets is presented in Table 5.25. The bulk density and tapped density of the domperidone CR pellets were found to be in the range of 0.79 to 0.86 gm ml^{-1} and 0.81 to 0.88 gm ml^{-1} respectively whereas for venlafaxine pellets, bulk and tapped densities were found to be in the range of 0.69 to 0.72 gm ml^{-1} and 0.70 to 0.75 gm ml^{-1} respectively. Densities of domperidone pellets were found to be higher than venlafaxine pellets because domperidone pellets contains high density material i.e. DCP. The observed values of Hausner's ratio for all pellet batches were in the range of 1.003 to 1.037 which indicates good flow properties of the developed pellets. Furthermore, % Carr's index values for all pellet formulations were found to be less than 3.63% which demonstrated excellent flow properties of designed formulations. Furthermore, faster flow rates for domperidone pellets and venlafaxine pellets were observed in the range of 3.37 to 3.77 gm s^{-1} and 4.20 to 4.77 gm s^{-1} respectively which further demonstrated good roundness of the pellets.

5.3.1.5.3. Friability, crushing strength and residual moisture content studies

Results of friability, crushing strength and residual moisture content studies are summarized in Table 5.26. Highest friability value was observed for the pellet batch DOM/IR/05 compared to other batches which might be attributed to lack of Carbopol which also acts binder. Pellet formulations with higher amount of Carbopol showed lower friability. However, all pellet formulations showed acceptable friability values (≤ 1% w/w). Further, crushing strength of pellets was also determined to evaluate mechanical strength of pellets. Crushing strength values for selected Carbopol based pellet formulations were observed in the range of 13.50 to 22.23 N.

Table 5.24. Summary for %yield, size and shape analysis data ((each value is result of nine separate determinations)

Formulation batch	Geometric mean diameter (d_g, mm) (Mean ± SD)	Span (Mean ± SD)	% Yield (Mean ± SD)	Shape analysis	
				Roundness (Mean ± SD)	Elongation (Mean ± SD)
DOM/PS/13	1.516 ± 0.008	0.727 ± 0.004	58.13 ± 1.34	0.613 ± 0.065	1.515 ± 0.261
DOM/PS/17	1.517 ± 0.009	0.707 ± 0.005	55.71 ± 2.12	0.665 ± 0.075	1.491 ± 0.198
DOM/IR/05	1.557 ± 0.007	0.755 ± 0.007	64.42 ± 2.23	0.729 ± 0.054	1.369 ± 0.112
DOM/BBD/OB/1	1.557 ± 0.006	0.523 ± 0.005	89.56 ± 1.56	0.936 ± 0.052	1.068 ± 0.068
VEN/PS/06	1.011 ± 0.005	0.748 ± 0.005	68.37 ± 1.23	0.635 ± 0.089	1.495 ± 0.162
VEN/PS/08	1.015 ± 0.004	0.769 ± 0.002	51.56 ± 2.83	0.674 ± 0.054	1.521 ± 0.198
VEN/BBD/OB/1	1.006 ± 0.005	0.516 ± 0.002	90.70 ± 1.11	0.912 ± 0.037	1.059 ± 0.054
VEN/BBD/OB/2	1.018 ± 0.006	0.511 ± 0.006	91.12 ± 1.05	0.893 ± 0.052	1.093 ± 0.065

Table 5.25. Summary for flow properties data (each value is result of nine separate determinations)

Formulation batch	Bulk density (gm ml^{-1}) (Mean ± SD)	Tapped density (gm ml^{-1}) (Mean ± SD)	Hausener's ratio (Mean ± SD)	% Carr's index (Mean ± SD)	Flow rate (gm s^{-1}) (Mean ± SD)
DOM/PS/13	0.79 ± 0.001	0.81 ± 0.001	1.027 ± 0.002	2.70 ± 0.150	3.37 ± 0.153
DOM/PS/17	0.81 ± 0.001	0.83 ± 0.002	1.028 ± 0.004	2.79 ± 0.408	3.51 ± 0.338
DOM/IR/05	0.85 ± 0.013	0.88 ± 0.003	1.037 ± 0.013	3.57 ± 1.240	3.73 ± 0.209
DOM/BBD/OB/1	0.86 ± 0.001	0.88 ± 0.007	1.028 ± 0.006	2.77 ± 0.600	3.77 ± 0.208
VEN/PS/06	0.72 ± 0.001	0.75 ± 0.001	1.031 ± 0.002	3.06 ± 0.137	4.13 ± 0.116
VEN/PS/08	0.69 ± 0.002	0.70 ± 0.001	1.003 ± 0.001	1.11 ± 0.264	4.20 ± 0.105
VEN/BBD/OB/1	0.71 ± 0.002	0.73 ± 0.002	1.025 ± 0.005	2.46 ± 0.544	4.56 ± 0.115
VEN/BBD/OB/2	0.71 ± 0.002	0.74 ± 0.012	1.037 ± 0.017	3.63 ± 1.577	4.71 ± 0.110

Formulation batches viz. DOM/PS/13, DOM/PS/17 and DOM/BBD/OB/1 showed higher crushing strength which might be attributed to higher amount of Carbopol 971 and presence of DCP in these formulations. These observed crushing strength values confirmed that the mechanical strength of designed pellets needed to withstand the stress generated during further processing and handling.

The residual moisture content in case of all batches of pellet formulations were found to be ≤ 1.23% which showed that selected conditions for drying were optimum.

Table 5.26. Summary for mechanical strength and residual moisture content (each value is result of nine separate determinations)

Formulation batch	Friability (% w/w) (Mean ± SD)	Crushing strength (N) (Mean ± SD)	Residual moisture content (%) (Mean ± SD)
DOM/PS/13	0.47 ± 0.090	19.76 ± 0.351	1.23 ± 0.052
DOM/PS/17	0.44 ± 0.051	20.57 ± 1.379	0.97 ± 0.115
DOM/IR/05	0.81 ± 0.044	10.83 ± 0.251	0.88 ± 0.189
DOM/BBD/OB/1	0.35 ± 0.042	22.23 ± 0.585	1.09 ± 0.075
VEN/PS/06	0.56 ± 0.050	16.90 ± 0.953	0.96 ± 0.005
VEN/PS/08	0.73 ± 0.047	16.67 ± 1.504	1.12 ± 0.068
VEN/BBD/OB/1	0.61 ± 0.017	14.30 ± 0.755	0.94 ± 0.172
VEN/BBD/OB/2	0.60 ± 0.049	13.50 ± 0.824	0.98 ± 0.230

5.3.1.5.4. Drug content studies

CR pellets of domperidone contained drug in the range of 98.85 to 99.71% (Table 5.27). Whereas, venlafaxine pellets contained drug in range of 98.67 to 99.56% (Table 5.28) respectively. These values indicated that the assay results were satisfactory. Also, low %RSD values in assay results indicated that the uniform mixing was achieved during the formulation of pellets.

Table 5.27. Drug content study for domperidone pellets (each value is result of nine separate determinations)

Formulation batch	% Assay ± SD; %RSD
DOM/PS/13	99.39 ± 0.34; 0.34
DOM/PS/17	98.95 ± 0.73; 0.73
DOM/IR/05	99.10 ± 0.34; 0.34
DOM/BBD/OB/1	99.71 ± 0.86; 0.86

Table 5.28. Drug content study for venlafaxine pellets (each value is result of nine separate determinations)

Formulation batch	% Assay ± SD; %RSD
VEN/PS/06	99.39 ± 0.78; 0.78
VEN/PS/08	99.56 ± 0.42; 0.42
VEN/BBD/OB/1	98.67 ± 0.66; 0.66
VEN/BBD/OB/2	98.90 ± 0.21; 0.21

5.3.1.5.5. In vitro release studies

In case of domperidone pellets, in vitro release studies were performed for optimized formulation batch i.e. DOM/BBD/OB/1. Also, similar formulation batch without Carbopol 971 (DOM/IR/05) was prepared and compared its release profile with DOM/BBD/OB/1. Release profiles for both formulations is shown in Figure 5.11. MDT and $t_{80\%}$ values for DOM/BBD/OB/1 were found to be 5.49 hr and 13.80 hr respectively whereas MDT and $t_{80\%}$ values for DOM/IR/05 were found to be 1.21 hr and 2.99 hr. These higher MDT and $t_{80\%}$ values for DOM/BBD/OB/1 indicates that addition of Carbopol 971 has extended the drug release from domperidone pellets significantly.

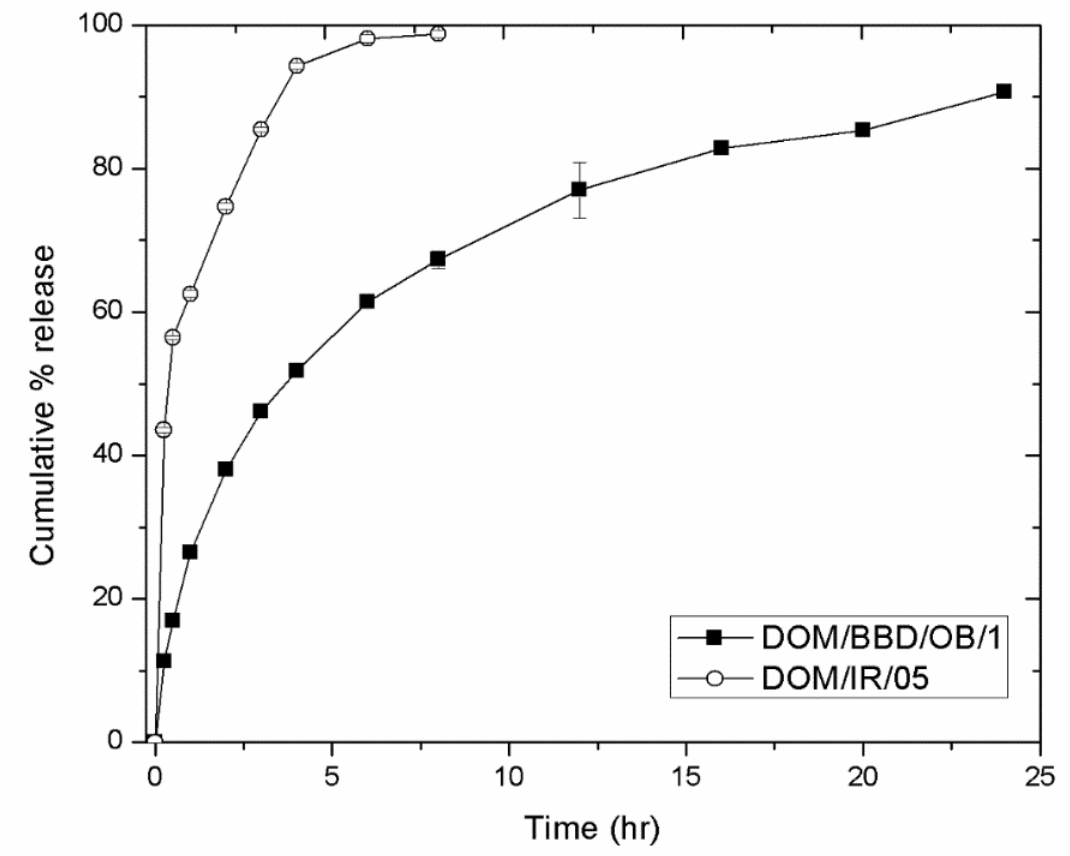

Figure 5.11. In vitro drug release profile for DOM/BBD/OB/1 (pellets with Carbopol 971) and DOM/IR/05 (pellets without Carbopol 971)

Additionally, release data of optimized pellet formulation of domperidone i.e. DOM/BBD/OB/1 was fitted to the various dissolution models. Observed results for various dissolution models are represented in Table 5.29. Currently, most used criteria in the field of dissolution model identification are the $R^2_{adjusted}$, Akaike information criterion (AIC), and the model selection criterion (MSC) [48-51]. Model with highest $R^2_{adjusted}$, lowest AIC value and MSC value of more than three was considered as best fit model [28,2,4]. So, Baker-Lonsdale model was found to be best fit model. It is modified form of Higuchi model and it describes the drug release from spherical matrix. Release exponent (n) value calculated from Peppas model was found to be 0.372 which indicates that domperidone released from Carbopol based matrix pellets with Fickian diffusion mechanism.

Table 5.29. Summary of various dissolution modeling data for in vitro release profile of domperidone CR matrix pellet formulation (DOM/BBD/OB/1)

Dissolution models	Dissolution rate constant (k)	$R^2_{adjusted}$	AIC	MSC
Zero order	4.979	0.1907	107.60	0.0449
First order	0.163	0.9004	82.46	2.1398
Higuchi	21.149	0.9151	80.54	2.3000
Korsmeyer-Peppas	29.227	0.9767	65.87	3.5220
Hixson-Crowell	0.046	0.8248	89.24	1.5700
Hopfenberg	0.000	0.8904	84.46	1.9700
Baker-Lonsdale	0.014	0.9927	51.12	4.7500

In case of venlafaxine, dissolution studies performed for VEN/BBD/OB/1. The relase profile is shown in Figure 5.12. MDT and $t_{80\%}$ values for VEN/BBD/OB/1 were found to be 5.64 min and 7.64 min respectively which indicates rapid drug releasing nature of formulation. So, these venlafaxine pellet formulations can be directly used as IR formulations or it can be further processed using powder layering or fluidized bed technology for modified release formulations.

5.3.1.5.6. Batch reproducibility

Batch to batch reproducibility of the manufacturing process used for the preparation of Carbopol pellets was investigated based on evaluation of the physical properties and in vitro release properties from three independently prepared batches of few selected domperidone and venlafaxine hydrochloride pellet formulations. All evaluations were carried in triplicate.

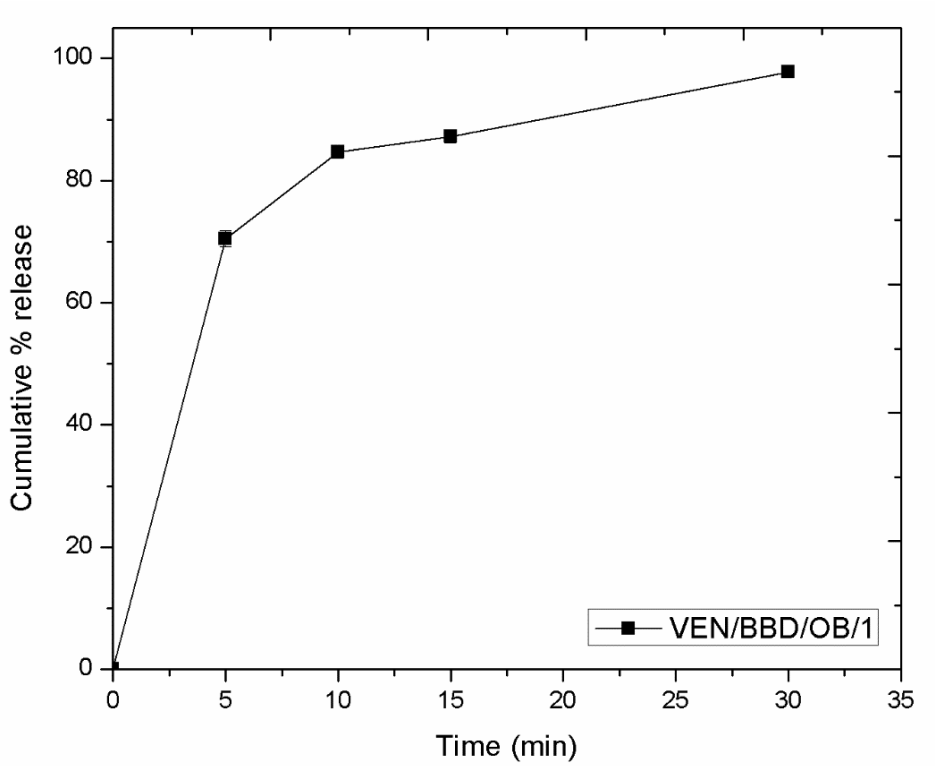

Figure 5.12. In vitro drug release profile for venlafaxine hydrochloride pellet formulation (VEN/BBD/OB/1)

Lower values of standard deviation for assay, size analysis, flow properties and crushing strength studies for three independently prepared batches, demonstrated that the manufacturing process employed was reliable and reproducible (Table 5.24 to 5.28). The f_2 values in all the cases found to be more than 85 indicated that there was no significant difference in drug release profiles of three independently prepared batches, which also confirmed excellent batch to batch reproducibility for designed pellet formulations.

5.3.1.5.7. Stability studies of formulations

Table 5.30 summarizes the results of stability studies carried out on the designed formulations at different condition of temperature and humidity. At refrigerated condition (FT: 5 ± 2 ºC), all the formulations samples were found to be stable for entire study period (12 month). So, the data has not been given for this condition. The log %RTD versus time profiles were linear for the formulations of both drugs at various storage conditions which indicates first order degradation kinetics.

At accelerated condition (AT: 40 ± 2 ºC / 75 ± 5% RH)**,** pellet formulations of domperidone and venlafaxine hydrochloride were found stable with $t_{90\%}$ values in the range of 25.68 to 28.07 and 25.09 to 28.79 months respectively. Formulations of both

drugs were found to be stable for entire study duration (6 months) with no apparent change in physical characteristics.

Similarly, in the formulations stored at controlled room temperature (CRT: 25 ± 2 ºC / 60 ± 5% RH), both domperidone and venlafaxine hydrochloride showed acceptable stability with $t_{90\%}$ values of 51.09 to 55.57 and 51.34 to 54.80 months respectively. All the formulations were stable for entire study duration (12 months) with no apparent change in physical characteristics.

The in-vitro release profiles of all the formulations samples) stored at long-term storage and accelerated storage condition were compared with the release profiles of zero-time samples (data not given). The f_2 factor values in all the cases found to be more than 75 which indicates that release profile from the stability samples was similar to initial (zero-time) profiles for all formulations.

Table 5.30. Stability data for domperidone and venlafaxine hydrochloride pellets formulations stored at long term storage conditions and accelerate storage conditions

Formulation batch	Long term storage condition: 25 ± 2 ºC /60 ± 5% RH			Accelerated storage condition: 40 ± 2 ºC / 75 ± 5% RH		
	K_{deg} (month^{-1})	r^2	$t_{90\%}$ (month)	K_{deg} (month^{-1})	r^2	$t_{90\%}$ (month)
DOM/PS/13	0.001971	0.9342	53.45	0.003754	0.9448	28.07
DOM/PS/17	0.001896	0.9467	55.57	0.003892	0.9741	27.07
DOM/IR/05	0.002063	0.9741	51.09	0.004106	0.9218	25.66
DOM/BBD/OB/1	0.001922	0.9552	54.83	0.004102	0.9055	25.68
VEN/PS/06	0.001923	0.9367	54.80	0.004373	0.9742	25.09
VEN/PS/08	0.002052	0.9414	51.34	0.004076	0.9853	25.85
VEN/BBD/OB/1	0.001916	0.9261	54.99	0.003851	0.9527	27.37
VEN/BBD/OB/2	0.002068	0.9122	50.94	0.003659	0.9683	28.79

5.4. Conclusion

In the present study, controlled release matrix pellets of domperidone were successfully developed and optimized using extrusion-spheronization technology. Similarly, high drug loaded (up to 60% w/w) pellets of venlafaxine hydrochloride immediate release pellets were prepared and optimized. The developed pellet formulations of both drugs were found to possess acceptable physical characteristics indicating suitability of preparation technique adopted. The assay values and in vitro release studies of all the

designed formulations were found to be highly satisfactory. The developed pellet formulations for both domperidone and venlafaxine hydrochloride were found to be stable during stability studies designed as per ICH and WHO guidelines. These developed carbopol based pellet formulations prepared using extrusion-spheronization technology can overcome many disadvantages associated with conventional pellet manufacturing processes such as powder layering method, fluidized bed processor, etc. Further, methods used for pellet preparation was found to be relatively simple and can easily be adopted for the development on a commercial scale.

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6. Pharmacokinetic Studies

6.1. Introduction

The primary goal of any drug delivery system is to provide an effective therapeutic concentration of drug at the site of action for the intended duration to produce desired pharmacological effects [1-2]. So, it is also essential to conduct pharmacokinetic and bioavailability studies to predict the therapeutic efficacy for any newly developed formulations. Pharmacokinetics study using animal models with suitable bioanalytical method provides fast and reliable results which offer an insight into the in vivo fate of the drug and its efficacy. These preclinical pharmacokinetic studies in animals done at an early stage of product development as proof of concept and to provide guidance for designing human trails [3-4]. In the present research work, it is proposed to design and develop a controlled release multiparticulate dosage form using Carbopol polymers. Thus, it is necessary to study the effect of Carbopol polymer on in vivo drug release behavior of developed formulation.

This chapter presents in vivo oral pharmacokinetic studies carried out for in-house developed domperidone pellets using Carbopol polymers with CPCSEA approved internal animal ethical committee clearance. Also, pharmacokinetic parameters of pellets with Carbopol and pellets without Carbopol were compared.

6.2. Experimental

6.2.1. Material and chemicals

Domperidone (assay ≥ 99.78%) was purchased from Lee Pharma Ltd., India. HPLC grade acetonitrile (assay > 99.7%), HPLC grade methanol (assay > 99.7%), potassium dihydrogen orthophosphate (assay > 99.5%) and orthophosphoric acid (assay ≥ 85%) were purchased from Merck Ltd., India. Diethyl ether was purchased from SD Fine-Chem Ltd., India. Standard animal diet was procured form Ratatouille Solutions Pvt. Ltd., Alwar, Rajasthan, India.

6.2.2. Animals used

Healthy male Wistar rats (180-220 g) were used for the pharmacokinetic study. Animals were procured from Central Animal Facility, BITS Pilani, India. Rats were housed in standard laboratory conditions. The animals had free access to standard diet and water. All animal care and experiments were conducted with prior approval of Institutional Animal Ethics Committee (IAEC) of Birla Institute of Technology and Sciences, Pilani, Rajasthan, India (Protocol approval numbers IAEC/RES/18/12, IAEC/RES/18/12/Rev-1/19/31).

6.2.3. Formulations for animal study

A fresh batch of controlled release (CR) pellet formulation of domperidone (DOM/BBD/OB/1) was prepared before the pharmacokinetic experiment. Similarly, a similar pellet formulation without Carbopol 971 was prepared (DOM/IR/05) which is considered as immediate release (IR) pellets. Further, all the quality control tests enlisted in chapter 5 were carried out for freshly prepared IR and CR pellets before proceeding with animal dosing. Further, domperiodne supesion was prepared with 0.5% w/w sodium carboxymethyl cellulose.

6.2.4. Administration of pure drug and formulations

Animals were kept on fasting overnight with free access to water before initiation of the study. Rats were randomly selected and divided in to three groups each consisting 8 rats. Number of animals required for the study was calculated using GPower software (GPower 3.1.9.2, Dusseldorf University and Kiel University, Germany). Suspension of domperidone (mg equivalent to the 10 mg kg^{-1}) was orally administered to the first group of rats [5]. The second group of rats orally received IR pellets (DOM/IR/05) whereas the third group of rats received CR pellets (DOM/BBD/OB/1) at the same dose as a pure drug.

6.2.5. Sample preparation

Around 0.5 ml of blood samples were collected from each rat (anaesthetized in diethyl ether chamber) through retro-orbital plexus at 0.5, 1, 2, 4, 6, 9, 12, 15, 24, 36 and 48 hr post-dosing of the formulations. The blood samples were processed to separate plasma as suggested in chapter 3 (Section 3.6.5). The plasma samples were analyzed using in-house developed bioanalytical HPLC method as described in chapter 3 (Section IC).

6.2.6. Data analysis

The plasma drug concentration at various time points was analyzed and subjected to non-compartmental analysis using Phoenix WinNolin Certera^{TM} software (Version 8.0; Pharsight Corporation, USA) to obtain various pharmacokinetic parameters such as concentration maximum (C_{max}), area under curve (AUC_{0-24}, $AUC_{0-\infty}$), mean residence time (MRT) and elimination half-life ($t_{1/2}$). Relative bioavailability (Fr) values for the CR pellets was determined as the ratio of $AUC_{0-\infty}$ of the CR pellets to the $AUC_{0-\infty}$ of IR pellets. Finally, results of oral pharmacokinetic studies were assessed using unpaired t tests with $P < 0.05$ level of significance.

6.3. Results and discussion

A pharmacokinetic study was carried out for domperidone administered orally in the form of suspension, IR pellets and CR pellets. The plasma concentration versus time profiles of these formulations are shown in Figure 6.1. AUC is the indicator of the extent of absorption and therefore it is an important parameter for analysis in a comparative bioavailability study. Thus, AUC for all formulations was determined. Further, C_{max}, T_{max}, MRT and $t_{1/2}$ were also determined as they are also important pharmacokinetic parameters for comparison as shown in Table 6.1.

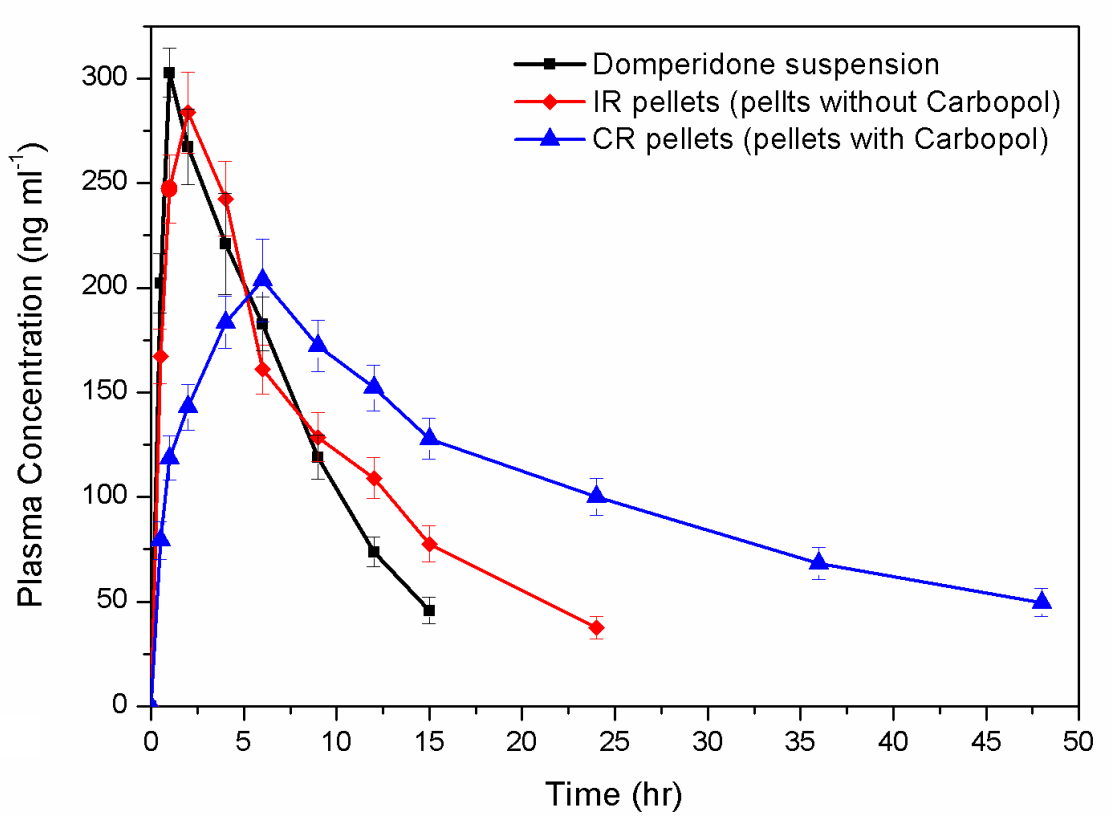

Figure 6.1. Comparative in vivo plasma drug concentration profile of drug suspension, IR and CR pellets of domperidone in rats after oral administration.

On administration of oral suspension, domperidone showed C_{max} of 302.83 ± 39.06 ng ml^{-1} at 1 hr (Table 6.1). Whereas, C_{max} and T_{max} values observed for IR pellets were found to be 283.68 ± 12.76 ng ml^{-1} and 2 hr respectively. In addition, MRT values for domperidone in case of oral suspension and IR pellets were found to be 7.25 hr and 12.60 hr respectively. A slight increase in T_{max} and MRT values in case of IR pellets compared to oral suspension can be attributed to the presence of Avicel PH101 and di-calcium phosphate (DCP) in pellets which might have resulted in the delayed disintegration of IR pellets [6-7].

Table 6.1. Pharmacokinetic parameters obtained for domperidone following oral administration of drug suspension, IR and CR pellets in rats (N=8).

Parameters	Oral suspension	IR pellets	CR pellets
C_{max} (ng ml^{-1})	302.83 ± 39.06	283.68 ± 12.76	223.53 ± 19.65
T_{max} (hr)	1	2	6
$AUC_{(0-\infty)}$ (ng hr ml^{-1})	2569.1 ± 235.18	3386.9 ± 125.18	6666.4 ± 898.18
$AUMC_{(0-\infty)}$ (ng hr^2 ml^{-1})	19333.8 ± 867.3	42070.6 ± 767.3	224403.9 ± 6457.3
MRT (hr)	7.25 ± 0.38	12.60 ± 0.85	34.96 ± 1.22
$t_{1/2}$ (hr)	4.58	8.82	23.04
Clearance (ml hr^{-1} kg^{-1})	3886.19 ± 78.12	2917.19 ± 65.87	1496.50 ± 67.74
Relative bioavailability	-	-	2.56

Pharmacokinetic profiles of domperidone obtained following administration of CR pellets were compared with that of IR pellets. The mean AUC value for the IR pellets was found to be 3386.9 ± 125.18 ng hr ml^{-1} and for CR pellets found to be 6666.4 ± 898.18 ng hr ml^{-1}. Higher $AUC_{0-\infty}$ values (Table 6.1) were observed following administration of CR pellets as drug released slowly and absorbed over longer duration of time. The difference was found to be statistically significant ($p < 0.5$). In addition, lower C_{max} and higher T_{max} values (Table 6.1) were observed for domperidone in case of CR pellets in comparison to the IR pellets and oral suspension. Further, MRT values for CR pellets (34.96 ± 1.22 hr) were found to be significantly higher than the MRT values obtained for IR pellets (12.60 ± 0.85 hr). Higher AUC values, increased MRT and higher T_{max} values suggest sustained drug release form for Carbopol based CR pellets. The $t_{1/2}$ is calculated from MRT and found to be higher for CR pellets as plasma drug concentration sustained over longer period. Similar difference in pharmacokinetic parameters were observed by many researchers during in vivo comparative studies between immediate and sustained release formulations [8-9].

6.4. Conclusion

The pharmacokinetic studies for the selected CR pellet formulation (pellets with Carbopol), IR pellet formulation (pellets without Carbopol) and oral suspension were conducted in rats. In vivo performance of CR pellet formulation was compared against an IR pellet formulation. The delayed T_{max}, reduced C_{max} and prolonged $t_{1/2}$ confirmed

controlled release character of the designed Carbopol based CR pellet formulation of domperidone.

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7. Conclusions and Future Prospective

7.1. Conclusions

Primary objective of the present research work was to explore the usefulness of Carbopol polymers for pellet preparation using extrusion-spheronization technology. If found successful, the process can be simpler, faster and cheaper for the preparation of pellets with extended release profile for many drugs. So, pellets were prepared using oral grades of Carbopol polymers (Carbopol 971 and Carbopol C974) with extrusion-spheronization technology without addition of any electrolytes. As the solubility of a drug has a substantial impact on designing an extended release formulation as well as the extrusion-spheronization process, domperidone (low water soluble) and venlafaxine hydrochloride (high water soluble) were selected as model drugs to explore the wide range applicability of Carbopols in this process.

As per the need of present research work, UV spectroscopic methods and stability indicating liquid chromatographic methods were developed for selected drugs and validated according to regulatory guidelines. These analytical methods were used for the estimation of drugs during preformulation and formulation studies. In addition, the bioanalytical method was also developed for the estimation of domperidone in rat plasma using solid phase extraction technique. Further, developed bioanalytical method was successfully applied for the estimation of domperidone during in vivo pharmacokinetic studies of the pure drug and pellet formulations in rats.

Prior to the formulation development, various preformulation studies including solubility, stability and drug-excipient compatibility studies were performed. Solubility studies demonstrated that domperidone exhibited a pH dependent solubility having higher solubility in acidic pH and low solubility in basic pH. On the other hand, venlafaxine hydrochloride exhibited pH independent solubility. Solution state stability studies suggested that both domperidone and venlafaxine hydrochloride followed first order degradation and were found to be stable in both acidic and basic pH conditions. The solid state stability studies confirmed that both the drugs are stable at refrigerated and ambient temperature. Drug-excipient compatibility studies revealed no significant interaction with selected excipients indicating their suitability for use in the formulation development.

Controlled release matrix pellets of domperidone were designed and developed using Carbopol polymers with extrusion-spheronization technology without the addition of

any electrolytes. More than 85% yield was achieved with suitable shape and size distribution. The drug release was extended up to 24 hr. Furthermore, in vitro release profile of these newly developed matrix pellets was successfully matched with domperidone pellets prepared using fluidized bed technology. High drug loaded pellets (up to 60% w/w) were also prepared for venlafaxine hydrochloride using Carbopol polymers with more than 90% yield. Various formulation variables and process parameters involved in extrusion-spheronization to develop Carbopol based pellets were screened using fractional factorial designs. Also, optimization studies were carried out using Box-Behnken design studies. The optimized pellets of both the drugs were found to possess acceptable physical characteristics indicating the suitability of the preparation technique adopted. The assay values and in vitro release studies of all the designed formulations were found within limits. The designed pellet formulations for both domperidone and venlafaxine hydrochloride were found to be stable during stability studies designed as per ICH and WHO guidelines.

Pharmacokinetic studies in rats were carried out for domperidone, administered orally in the form of suspension, immediate release pellets (pellets without Carbopol 971) and controlled release pellets (pellets with Carbopol 971). In vivo performance of controlled release pellet formulation was compared against an immediate release pellet formulation. The delayed T_{max}, reduced C_{max} as well as prolonged MRT and $t_{1/2}$ confirmed controlled release character of the designed Carbopol based pellets formulation of domperidone.

The results obtained by current research work indicated that Carbopol based pellets can be successfully prepared using extrusion-spheronization without addition of electrolytes. This Carbopol based controlled release pellet preparation method is simple, cost-effective, operator independent and requires very less amount of time compared to currently used methods. Also, it is a safer and greener process as it does not require the use of organic solvents and produces very low amount of fines/dust. To conclude, outcome of the present research work can be utilized in industry for economic product development.

7.2. Future Perspectives

Current study also advocates significant future scope of work, wherein, scale-up studies of the manufacturing process from lab scale to industrial scale must be performed and

optimized. More studies can be performed to determine granulation fluid required during wet mixing using mixer torque rheometer (MTR). Also, the effect of the ratio of length and diameter of the extrusion screen holes (L/R ratio) on final pellets quality such as shape and size distribution can be studied which may help us to improve yield further. Further, preparation Carbopol pellets using different types of extruders should be studied. This research work may be further extended for several drugs of different chemical nature with various solubility profiles to ensure wider applicability of Carbopol polymers in extrusion-spheronization. In case of domperidone pellets, in vitro/in vivo correlation (IVIVC) experiments and bioequivalence studies in human volunteers need to be performed in order to commercialize the formulation.

8. Appendix

List of Publications and Presentations

Publications based on thesis

1. **S. Reddi**, G. Shingvi, A. Khosa, V. Krishna, R. Saha "Development and validation of a sensitive and stability indicating HPLC method for the determination of venlafaxine in bulk and formulations" ***Current Pharmaceutical Analysis***, 2018, 14:1-8.
2. **S. Reddi**, G. Shingvi, A. Khosa, R. Saha "Development and validation of cost effective UV-spectrophotometric method for the estimation of domperidone in bulk and pharmaceutical formulations" ***Asian Journal of Chemistry***, 2017, 29: 2623-2626.
3. **S. Reddi**, S. Dubey, V. Krishna, A. Khosa, R. Saha "Development and validation of simple and sensitive HPLC-UV method for quantitative estimation of domperidone in rat plasma and its application in pharmacokinetic studies" ***International Journal of Analytical Chemistry***. *February 2018, under review.*
4. **S. Reddi**, A. Khosa, E. Joseph, R. Saha "Stability indicating RP-HPLC-UV method for domperidone with DoE centric robustness study and its applicability in drug-excipient compatibility and stability studies" ***Journal of Pharmaceutical and Biomedical Analysis***, *April 2018, communicated.*
5. **S. Reddi,** E. Joseph, A. Khosa, R. Saha "Development, optimization and in vivo evaluation of Carbopol based controlled release domperidone pellets prepared using extrusion-spheronization" ***Expert Opinion on Drug Delivery,*** *April 2018 communicated.*

Other publications

6. E. Joseph, **S. Reddi**, V. Rinwa, G. Balwani, R. Saha "DoE based Olanzapine loaded poly-caprolactone nanoparticles decreases extrapyramidal effects in rodent model" ***International Journal of Pharmaceutics*****.** 2018, 541:198-205.
7. A. Khosa, **S. Reddi**, R. Saha "Nanostructured lipid carriers for site-specific delivery" ***Biomedicine & Pharmacotherapy*****.** 2018, 103:598-613.
8. E. Joseph, **S. Reddi**, V. Rinwa, G. Balwani, R. Saha "Design and in vivo evaluation of solid lipid nanoparticulate system of olanzapine for acute phase treatment: investigations on antipsychotic potential and adverse effect" ***European Journal of Pharmaceutical Sciences*****.** 2017, 104:315-325.

9. G. Balwani, E. Joseph, V. Nagpal, **S. Reddi**, R. Saha "Rapid, simple and sensitive spectrofluorometric method for the estimation of ganciclovir in bulk and pharmaceutical formulations" ***Journal of Spectroscopy.*** 2013:1-5.
10. A. Khosa, S. Shelly, **S. Reddi**, R. Saha "Simple, rapid and sensitive UV spectrophotometric method for determination of temozolomide in poly-ε-caprolactone nanoparticles" ***Asian Journal of Chemistry.*** 2018, 30 (4), 868-872.
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12. A. Khosa, S. Dubey, V. Krishna, **S. Reddi**, R. Saha "A simple, high throughput RP-HPLC method for the determination of anti-cancer drug temozolomide in rat plasma: validation and application to a pharmacokinetic study" ***Biomedical Chromatography.*** *February 2018, under review.*

Poster presentation

- **S. Reddi**, G. Shingvi, A. Khosa "Development and validation of stability indicating HPLC method for the estimation of domperidone in bulk drug and pharmaceutical formulations" International Conference on Challenges in Drug Discovery and Delivery (2017) held at BITS Pilani, Pilani Campus, Pilani.

Biography of Prof. Ranendra N. Saha

Dr. Ranendra Narayan Saha is Senior Professor of Pharmacy as well as Director of BITS Pilani, Dubai Campus. He is also a Senate and Research Board Member of BITS Pilani. Besides, he is a member of an advisory board and selection committee of several universities in India and overseas. He has completed his Bachelor of Pharmacy and Master of Pharmacy from Jadavpur University, Kolkata and Ph.D. from BITS, Pilani.

He has more than 34 years of teaching, research and administrative experience. His main research interests are in nanomedicine, oral solid dosage forms and pharmacokinetics. He is an author of over 100 research papers and presented more than 100 papers in conferences/seminars. He has also authored a book and several book chapters with renowned publishing houses. Besides, he has delivered several invited lectures at seminars/conferences organized by various colleges, pharmaceutical industries and universities in India and overseas. He has completed several government and industry sponsored projects. He has developed commercial products for industries, transferred technologies to industries and secured patents. He has supervised several doctoral, postgraduate and undergraduate students.

He received *'Pharmacy Professional of the Year 2013'* an award given by Indian Association of Pharmaceutical Scientists and Technologists (IAPST). In 2011, he has been awarded *Shri B. K. Birla and Shrimati Sarala Birla Chair Professorship* at BITS Pilani for his contributions in teaching and research. He is also a recipient of '*The Best Pharmacy Teacher Award'* for the year 2005, awarded by Association of Pharmaceuticals Teachers of India (APTI), in recognition of his contribution in teaching and research in the field of Pharmacy.

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Satish Sangappa Reddi is a Research Scholar at Department of Pharmacy, BITS Pilani, Pilani Campus. He is working on an industrial project sponsored by Lubrizol Advanced Material Pvt. Ltd., for pursuing doctoral research. He has completed his Bachelor of Pharmacy from Government College of Pharmacy, Karad (Shivaji University), Maharashtra in 2009 and Master of Pharmacy from AISSMS College of Pharmacy, Pune (Pune University), Maharashtra in 20011. He has working experience in tablets, pellets and nanocrystals. He has published research articles in renowned journals and presented papers in conferences.