Original Research Article

Quality risk management of top spray fluidized bed process for antihypertensive drug formulation with control strategy engendered by Box-behnken experimental design space

Amit Mukharya[1,2], Paresh U Patel[2], Dinesh Shenoy[2], Shivang Chaudhary[1]

[1]Formulation Development (F&D) Department, Regulated Market, Cadila Pharmaceuticals limited, Ahmedabad, [2]Quality Assurance (QA) Department, S. K. Patel college of Pharmaceutical, Education & Research, Ganpat University, Mehsana, India

Abstract

Introduction: Lacidipine (LCDP) is a very low soluble and highly biovariable calcium channel blocker used in the treatment of hypertension. To increase its apparent solubility and to reduce its biovariability, solid dispersion fluid bed processing technology was explored, as it produces highly dispersible granules with a characteristic porous structure that enhances dispersibility, wettability, blend uniformity (by dissolving and spraying a solution of actives), flow ability and compressibility of granules for tableting and reducing variability by uniform drug-binder solution distribution on carrier molecules. **Materials and Methods:** Main object of this quality risk management (QRM) study is to provide a sophisticated "robust and rugged" Fluidized Bed Process (FBP) for the preparation of LCDP tablets with desired quality (stability) and performance (dissolution) by quality by design (QbD) concept. **Results and Conclusion:** This study is principally focusing on thorough mechanistic understanding of the FBP by which it is developed and scaled up with a knowledge of the critical risks involved in manufacturing process analyzed by risk assessment tools like: Qualitative Initial Risk-based Matrix Analysis (IRMA) and Quantitative Failure Mode Effective Analysis (FMEA) to identify and rank parameters with potential to have an impact on In Process/Finished Product Critical Quality Attributes (IP/FP CQAs). These Critical Process Parameters (CPPs) were further refined by DoE and MVDA to develop design space with Real Time Release Testing (RTRT) that leads to implementation of a control strategy to achieve consistent finished product quality at lab scale itself to prevent possible product failure at larger manufacturing scale.

Key words: Critical process parameter, critical quality attribute, failure mode effective analysis, fluidized bed process, quality by design, quality risk management, scale-up.

INTRODUCTION

Lacidipine (LCDP) is a very low soluble and highly biovariable calcium channel blocker used in the treatment of hypertension. To increase its apparent solubility and to reduce its biovariability,

Address for correspondence:
Dr. Amit Mukharya,
Formulation Development (F and D) Department,
Regulated Market, Cadila Pharmaceuticals limited,
Ahmedabad - 387 810, India.
E-mail: amit.mukharya@cadilapharma.co.in

has been proven very much advantageous, as it produces highly dispersible granules with a characteristic porous structure that enhances dispersibility, wettability, blend uniformity (by dissolving and spraying a solution of actives), flow ability and compressibility of granules for tabletting. However, at the R and D stage; experiments are carried out in bench top or small pilot-scale equipment. The scale-up from laboratory equipment to production-size units is dependent on equipment design, which may or may not have been scalable as far as its selected dimensional features or components is concerned. Moreover, the fluid bed agglomeration process is a combination of three steps: dry mixing, spray agglomeration, and drying to a desired moisture level or to a desired granule size. Quality of the granules is determined during the spraying stage only where constant building of granules and evaporation of binder solvent is taking place.[1] Authors found that the critical processing factors that most affect granule characteristics were rate of binder addition,[2] degree of atomization of the binder liquid,[3] process-air temperature,[4] fluidization air velocity,[5]

and height of the spray nozzle from the bed.[6] Each of this process parameter is a very high risk for the development of robust and rugged fluid bed process (FBP), thus it should be optimized and managed properly in precise manner to reduce its impact on overall quality of end product irrespective of scale.[7-9] Quality risk management (QRM) is a systematic process for the assessment, control, communication, and review of any process-related risks to the quality of the drug product across the product lifecycle. An ideal model for QRM is outlined in the Figure 1.

A) **Risk assessment** consists of the identification of hazards and the analysis and evaluation of risks associated with exposure to those hazards. Quality risk assessments begin with a well-defined problem description or risk question. As an aid to clearly defining the risk(s) for risk assessment purposes, three fundamental questions are often helpful:

- What might go wrong?
- What is the likelihood (probability) it will go wrong?
- What are the consequences (severity)?

Risk identification addresses the "What might go wrong?" question, including identifying the possible consequences. Information can include historical data, theoretical analysis, informed opinions, and the concerns of stakeholders.
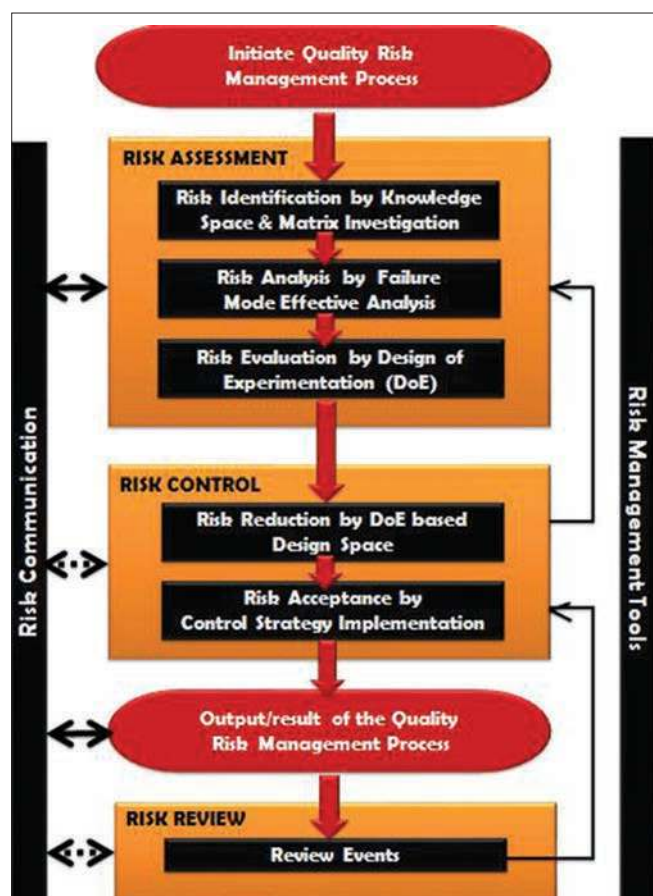

Figure 1: Overview of a typical quality risk management process

Risk analysis is the estimation of the risk associated with the identified hazards. It is the qualitative or quantitative process of linking the likelihood of occurrence and severity of harms with the ability to detect the harm (detectability).

Risk evaluation is a quantitative estimate of risk through multivariate data analysed by conducting series of Design of Experiments (DoEs) for evaluating actual significant effect of individual factor and interaction factors on response in test. When risk is expressed quantitatively, a numerical probability is used. Alternatively, risk can be expressed using qualitative descriptors, such as "high", "medium", or "low".

B) **Risk control** includes decision making to reduce and/or accept risks. The purpose of risk control is to reduce the risk to an acceptable level. Risk control might focus on the following questions:
- Is the risk above an acceptable level?
- What can be done to reduce or eliminate risks?
- What is the appropriate balance among benefits, risks, and resources?

Risk reduction might include actions taken to mitigate the severity and probability of harm by implementing design space (DS)-based control strategy. Processes that improve the detectability of hazards [i.e. Process Analytical Technology (PAT) tools for Real Time Release Testing (RTRT)] might also be used as part of a risk control strategy.

Risk acceptance can be a formal decision to accept the residual risk.

C) **Risk communication** is the sharing of information related to the existence, nature, form, probability, severity, acceptability, control, treatment, detectability, or other aspects of risks between the industry and regulators.

D) **Risk Review:** The output/results of the risk management process should be reviewed to take into account of new knowledge and experience.

Thus, main object of this QRM study is to provide a sophisticated robust and rugged FBP for the preparation of LCDP Tablets with desired quality (stability) and performance (dissolution) by Quality by Design (QbD) concept. This study is principally focusing on thorough understanding of the process by which it is developed and scaled up with a knowledge of the critical risks involved in manufacturing process analyzed by Risk assessment tools like: IRMA (Initial Risk-based Matrix Analysis) and FMEA (Failure Mode Effective Analysis) to identify and rank parameters with potential to have an impact on In Process/Drug Product Critical Quality Attributes (IP/DP CQAs), based on prior knowledge and initial experimental data; which were refined further to determine the significance of individual variables and interactions by developing DS with DoE and MVDA for critical process parameters that leads to mechanistic understanding for individual critical process parameter (s) to implement a control strategy to achieve consistent finished product quality at pilot-scale developmental stage itself

to prevent possible product failure at larger commercial manufacturing scale.

MATERIALS AND METHODS

Materials

LCDP was procured from Cadila Pharmaceuticals limited, India. Polyvinyl Pyrrolidone (Plasdone® K29/32) was purchased from ISP Technologies. Lactose Monohydrate (Pharmatose® 200M and DCL 11®) were purchased from DMV International used as an intragranular diluent cum powder substrate. Absolute Alcohol (Ethanol 99.6%v/v) was procured from CVKUSML, India. Magnesium Stearate of vegetable grade was purchased from Ferro Synpro. Film Coating material, Opadry® White was purchased from Colorcon Asia limited, India.

Experimental methods

LCDP is an, once-a-day, orally administered, 1, 4-dihydro pyridine derived "Calcium channel blocker", antihypertensive with an intrinsically slow onset of activity ensuing in a lack of reflex tachycardia with a long duration of action and a high degree of vascular selectivity.[10] But the quandary is that LCDP is a biopharmaceutics (BCS) class IV drug with low solubility and highly variable permeability presenting a challenge to the formulation scientists.[11] Thus, solvent evaporation by FBP was selected as a method of choice for formulation by Solid Dispersion; as it improves wettability with simultaneous increase in porosity of granules. Moreover it also decreases the crystalline structure of drug and promotes its conversion in to more soluble amorphous form.[12] Optimized formulation having desired disintegration and dissolution rate comprises of LCDP, carrier (PVP), diluent and lubricant; wherein the weight ratio of LCDP to carrier is 1:10, with definite intra-granular lactose (Pharmatose 200M) to extra-granular lactose ratio (DCL 11) of 80:20 and magnesium stearate with adjusted weight gain of 2%w/w film coating is mentioned in Table 1a. Moreover, as LCDP is highly variable drug product, thus fluidized bed processing parameters should be precisely controlled to produce intended robust product as per predefined QTPP.

Development of SD-FBP technology

At pilot scale, for FBP (Pam-Glatt®-GPCP2); LCDP was first dissolved in ethanol (99.6%v/v) with stirring at slow speed until a clear solution was obtained. In this solution, PVP-K29/32 was slowly added with continuous stirring until a clear yellow-colored solution was obtained. To carry out Top spray fluidized bed granulation, 40# sifted Lactose Monohydrate (Pharmatose-200M) was loaded in fluidized bed processor and granulated by spraying of drug carrier solution (LCDP-PVP K29/32) for moistening of lactose powder substrate using top spray mechanics on fluidized bed as per Table 1a, while peristaltic pump RPM, spray rate, and atomization air pressure were very slowly increased up to optimum and recorded intermittently in every 10 minutes.

Table 1a: Optimized LCDP formulation

Intragranular (IG)	mg/unit
Intragranular	
Lacidipine	4.00
Plasdone K29/32	40.00
Pharmatose 200M	204.80
Extragranular	
Pharmatose DCL11	50.45
Magnesium stearate	0.75
Unit Weight of core tablet (in mg.)	300.00
%Weight gain in coating	2
Unit weight of coated tablet (in mg.)	306.00

Table 1b: Fluidized bed process parameters

FBP Parameters	Limit
Inlet temperature	50±10ºC
Outlet temperature	40±10ºC
Product temperature	30±10ºC
Liquid spraying rate and atomization air pressure	To be recorded

FBP: Fluidized bed process

Table 1c: Compression parameters

Compression parameters	Limits
Target weight	300 mg
Thickness	5.1±0.1 mm
Hardness	50 to 70 Newton
Disintegration time	NMT 15 minutes

After completion of granulation, fluidized bed drying was carried out in the same FBP at parameters declared in Table 1b, until desired constant LOD specifically from 1.5 to 2.5% w/w at 105°C was achieved. Dried granules were sifted through 20# screen in mechanical sifter. Dried sifted granules were mixed in double cone blender for 5 minutes at 10 ± 2 RPM with 40# presifted spray-dried Lactose (Pharmatose DCL-11) and lubricated with 60# presifted magnesium stearate. Lubricated granules were compressed using 12.7 × 7.1-mm oval-shaped punches at parameters revealed in Table 1c in 16 station rotary tablet compression machine (RIMEK®). Film Coating was carried out at inlet temperature of 60 ± 10ºC with Opadry® white suspension in 24" Auto-coater (Ganscoater®) until desired weight gain was achieved.

Optimization of SD-FBP parameters as per enhanced QBD

According to ICH Q8 Guideline "Quality cannot be tested into products; quality should be built-in by design". In all cases, the product was designed to meet patients' needs and the intended product quality and performance. A more systematic enhanced QbD approach for development included incorporation of prior knowledge, results of studies using design of experiments (ICH Q8),[13] use of QRM (ICH Q9)[14] and use of knowledge management (ICH Q10)[15] throughout the lifecycle of the product. A greater understanding of the product and its manufacturing process created a basis for more flexible regulatory approaches. Thus, for pharmaceutical development of stable product with robust process by enhanced QbD approach included following steps in succession:
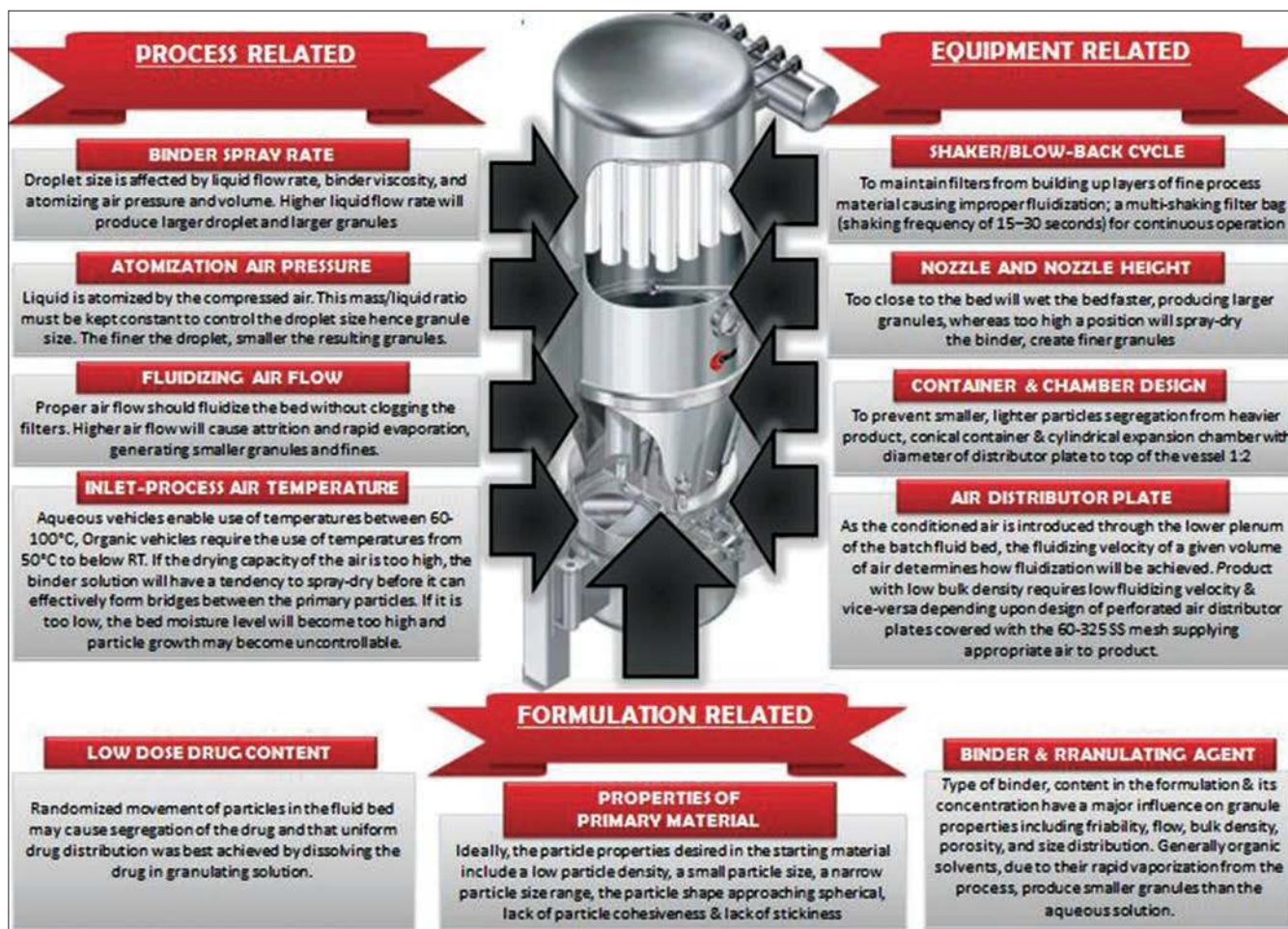
PROCESS RELATED

BINDER SPRAY RATE
Droplet size is affected by liquid flow rate, binder viscosity, and atomizing air pressure and volume. Higher liquid flow rate will produce larger droplet and larger granules

ATOMIZATION AIR PRESSURE
Liquid is atomized by the compressed air. This mass/liquid ratio must be kept constant to control the droplet size hence granule size. The finer the droplet, smaller the resulting granules.

FLUIDIZING AIR FLOW
Proper air flow should fluidize the bed without clogging the filters. Higher air flow will cause attrition and rapid evaporation, generating smaller granules and fines.

INLET-PROCESS AIR TEMPERATURE
Aqueous vehicles enable use of temperatures between 60-100°C, Organic vehicles require the use of temperatures from 50°C to below RT. If the drying capacity of the air is too high, the binder solution will have a tendency to spray-dry before it can effectively form bridges between the primary particles. If it is too low, the bed moisture level will become too high and particle growth may become uncontrollable.

EQUIPMENT RELATED

SHAKER/BLOW-BACK CYCLE
To maintain filters from building up layers of fine process material causing improper fluidization; a multi-shaking filter bag (shaking frequency of 15–30 seconds) for continuous operation

NOZZLE AND NOZZLE HEIGHT
Too close to the bed will wet the bed faster, producing larger granules, whereas too high a position will spray-dry the binder, create finer granules

CONTAINER & CHAMBER DESIGN
To prevent smaller, lighter particles segregation from heavier product, conical container & cylindrical expansion chamber with diameter of distributor plate to top of the vessel 1:2

AIR DISTRIBUTOR PLATE
As the conditioned air is introduced through the lower plenum of the batch fluid bed, the fluidizing velocity of a given volume of air determines how fluidization will be achieved. Product with low bulk density requires low fluidizing velocity & vice-versa depending upon design of perforated air distributor plates covered with the 60-325 SS mesh supplying appropriate air to product.

FORMULATION RELATED

LOW DOSE DRUG CONTENT
Randomized movement of particles in the fluid bed may cause segregation of the drug and that uniform drug distribution was best achieved by dissolving the drug in granulating solution.

PROPERTIES OF PRIMARY MATERIAL
Ideally, the particle properties desired in the starting material include a low particle density, a small particle size, a narrow particle size range, the particle shape approaching spherical, lack of particle cohesiveness & lack of stickiness

BINDER & RRANULATING AGENT
Type of binder, content in the formulation & its concentration have a major influence on granule properties including friability, flow, bulk density, porosity, and size distribution. Generally organic solvents, due to their rapid vaporization from the process, produce smaller granules than the aqueous solution.

Figure 2: Fluid bed process, equipment and formulation related factors affecting in process and/or finished product cqas.

Delineation of quality target product profile

The quality target product profile (QTPP) formed the basis of design for the development of the product. Considerations for the QTPP included intended use, route of administration, dosage form, strength, container closure system, attributes affecting pharmacokinetic characteristics, purity, and stability appropriate for the intended product.

Identification of critical quality attributes

A CQA is a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range or distribution to ensure the desired product quality. CQAs are generally associated with the drug substance, excipients, intermediates (in-process materials), and drug product. For drug substances, raw materials and intermediates, the CQAs can additionally include those properties (e.g. particle size distribution, density, and flow properties) that affect drug product CQAs. Potential drug product CQAs derived from the QTPP and/or prior knowledge was used to guide the product and process development. For drug product, CQAs of solid oral dosage forms are typically those aspects affecting product purity, strength, drug release and stability. The list of potential CQAs was modified when the formulation and manufacturing process were

selected and as product knowledge and process understanding increased. Relevant CQAs were identified and prioritized by an iterative process of QRM and experimentation that assessed the extent to which their variation had an impact on the quality of the drug product.

Critical quality risk analysis of critical process parameters by quality risk management

Risk assessment is a valuable science-based process used in QRM (ICH Q9) that aided in identifying which material attributes and process parameters potentially had an effect on product CQAs as represented in Figure 2. Risk assessment was typically performed early in the development stage and was repeated as more information and greater knowledge was obtained. Risk assessment tools i.e. matrix analysis and failure mode effective analysis were concisely used to identify and rank parameters with potential to have an impact on IP/DP CQAs, based on prior knowledge and initial experimental data. This list was refined further through experimentation to determine the significance of individual variables and potential interactions through a combination of DOEs, mathematical models or studies that lead to mechanistic understanding to achieve a higher level of process understanding.

Optimization of processing parameters and establishment of design space

Depending on IRMA and FMEA results, process understanding experiments [Design of Experiments (DoE) and Multi-Variate Data Analysis (MVDA)] were developed for FBP and Compression parameters having higher risk priorities i.e. more than 15 among all processes involved in product development. The effect of each independent CPPs on dependent product quality (e.g. average granule size and tablet hardness) were analyzed for establishment of DS to design, analyze, and control manufacturing through timely measurements of critical quality and performance attributes of raw and in-process materials, which were modeled out with the goal of ensuring product quality. Here, for establishment of DS for CPPs, full factorial 3^2 designs was used for optimization procedure, because it was suitable for investigating the quadratic response surfaces for constructing a second-order polynomial model, thus enabling optimization of liquid spraying rate and atomization air pressure to achieve desired average granule size i.e. NMT 400 μm without possibility of lump formation and to decide a desired range of tablet thickness at optimum turret speed to achieve anticipated hardness for prerequisite dissolution. Response surface methodology (RSM) was performed by engaging Design-Expert® software (Version 8.0, Stat-Ease Inc., Minneapolis, MN). Scaling factors were also included for DS intended to span multiple operational scales. Dimensionless numbers for scaling was included as part of the DS description.

Pivotal scale-up considering prior pilot scale QbD

Scale-up of FBP from small laboratory units to large commercial machines has been a continuing activity in pharmaceutical industry. Traditional approach of product development included limited development and scale-up work with final confirmation by validation of 3 batches at pivotal scale. Moreover, there was also a possibility of 'Worst-case' scenarios supposed to be included "Market recalls" and "underutilization of capacity" indicated limited success. While in QbD, complete understanding of product and process with monitoring, corrective and preventive actions of all critical steps were taken care at pilot-scale developmental stage to prevent product failure at larger scale. Henceforth, acceptable quality of the product would be ensured with "no recalls" and maximize utilization of capacity. Fluid bed scale-up is a mix of mathematics, engineering and personal judgment. Equipment variables, such as the type and size of the equipment and key process variables such as spray rate, atomization pressure, and inlet air temperature affect the product quality attributes. Control of such parameters to yield a consistent product at a large batch size, thereby constituted a successful scale-up strategy. Consistent quality of incoming raw material was also very important i.e., active pharmaceutical ingredients and excipients.

Among the three steps involved in fluid-bed agglomeration (dry mixing, spray agglomeration, and drying) the spray agglomeration stage was the most critical phase to monitor. During this phase, dynamic granule growth and breakdown takes place, along with solvent evaporation. Thus by QbD, risk associated with scale-up

was considered in control strategy of pilot-scale development itself to maximize the probability of effectiveness at larger scale with utilized QRM tools to guide activities. Proposed DS is subject to regulatory assessment and working within the space is not a change. Movement out of DS is considered to be a change and requires scale-up post-approval change process. The relevance of a DS developed at small or pilot scale to the proposed production scale manufacturing process was justified and discussed with the potential risks in the scale-up operation with predetermined edges of failure for process parameters or material attributes, beyond which the relevant quality attributes, could not be met.

RESULTS AND DISCUSSION

Pilot-scale process optimization by QbD
Definition of QTPP with reference to Ip/Dp CQAs

First, QTPP with reference to IP CQA and FP CQA was identified as it relates to quality, safety and efficacy, considering e.g., the route of administration, dosage forms, bioavailability, and stability as represented in Table 2.

Risk identification by knowledge space and matrix investigation of critical process variables affecting CQAs

A cross-functional team of authors has worked together for brainstorming to develop an Ishikawa (fishbone) diagram as represented in Figure 3 that identified potential formulation and FBP factors in knowledge which can have an impact on the desired quality attribute. The variables were then ranked qualitatively in Matrix Analysis (i.e., having low, medium, or high impact) based on prior knowledge and initial experimental data as mentioned in Table 3. Then after DoE was used to evaluate the impact of the higher ranked variables, to gain greater understanding of the process, and to develop a proper control strategy.
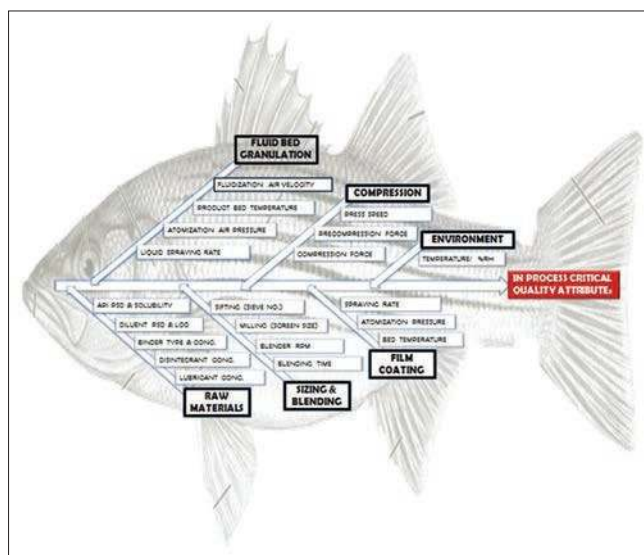
Figure 3: Ishikawa (Fish Bone) Diagram illustrating factors in knowledge for involved Processing steps affecting Finished Product Critical Quality Attributes (CQAs)

As compared to other processing steps (i.e., sizing, blending, compression, and film-coating) involved in the manufacturing of LCDP tablets, fluidized bed granulation is the most critical step involving high risk possessing scale-up parameters, which have high impact on one or more in-process and finished product CQA(s). i.e., (i) Liquid Spraying Rate: Higher liquid flow rate will produce larger droplet and larger granules (ii) Atomization Air Pressure: Higher pressure will produce finer droplet, resulting in smaller granules (iii) Fluidization Air Velocity: Higher air flow will cause attrition and rapid evaporation, generating smaller granules and fines. Proper air flow should fluidize the bed without clogging the filters, which can affect process efficiency in terms of %yield.

Risk assessment by failure mode effective analysis

Risk included severity of harm, probability of occurrence, and detects ability, and therefore the level of risk was evaluated quantitatively by FMEA in the term of Risk Priority Number as a result of QRM as summarized critically in Table 4.

Risk evaluation by generation of DoE based design space and multivariate data analysis

Depending on IRMA and FMEA results, process understanding experiments [Design of Experiments (DoE) and Multi-Variate Data Analysis (MVDA)] were developed for FBP having higher risk priorities i.e. more than 25. The effect of CPPs on product quality were analyzed by one of the RSM i.e. 3-level Box-Behnken Experimental Design for establishment of DS to design, analyze and control manufacturing through timely measurements of critical CPPs, which were modeled out with the goal of ensuring product quality in terms of CQAs as revealed in Table 5. The Box-Behnken design (BBD) utilized in this QRM study provides strong coefficient near the center of the DS, where the presumed optimum is located. 2D

Table 2a: Definition of quality target product profile with reference to In process critical quality attributes

IP CQAs	Quality target product profile
Appearance	White to off-white free flowing granules
Assay	95-105% of the label claim of composite blend sample
Blend uniformity	95-105% of the label claim for Individual blend sample.Mean value: 97-103%, acceptance value: NMT 15.0, RSD: NMT 5.0%
Average granule size	D50:NMT 400 μm
Bulk density	NLT 0.40 g/cc
Tapped density	NLT 0.50 g/cc
Carr's index	NMT 20
Hausners ratio	NMT 1.25
Angle of Repose	NMT 35°
%Loss of drying	NMT 2.0%w/w at 105°C/4 min

IP CQAs: In process critical quality attributes

Table 2b: Definition of quality target product profile with reference to finished product critical quality attributes

DP CQAs	Quality target product profile
Appearance	White to off-white, oval-shaped, coated tablets having embossed with "C" and "P" on one side with break line on both side.
Assay	95-105% of the label claim
Impurities	Impurity A: NMT 0.5%; Impurity B: NMT 2.0%; Any Other impurity: NMT 0.5%; Total impurities: 2.5%
Content Uniformity	Acceptance value: NMT 15.0 RSD: NMT 5.0%
Disintegration	Not more than 15 minutes
Dissolution	Not less than 75% (Q) of the labeled amount dissolved in 45 minutes

DP CQAs: Drug product critical quality attributes

Table 3a: Initial qualitative risk-based matrix analysis for critical process parameters affecting In process critical quality attributes

IP CQAs	Unit operations involved				
	FB Process	Sizing	Blending	Compression	Film coating
Appearance	High	Medium	Low	Medium	Medium
Assay	High	Low	Low	Low	Low
LOD	High	Low	Low	Low	Medium
Blend uniformity	High	Medium	Medium	Low	Low
Flow properties	High	Medium	Low	Low	Low

IP CQAs: In process critical quality attributes, FB: Fluidized bed

Table 3b: Initial risk-based matrix analysis for critical process parameters affecting finished product critical quality attributes

FP CQAs	Unit operations involved				
	FB Process	Sizing	Blending	Compression	Film coating
Appearance	High	Medium	Low	Medium	Medium
Assay	High	Low	Low	Low	Low
Impurities	High	Low	Low	Low	Medium
Content uniformity	High	Medium	Medium	Low	Low
Dis integration	High	Low	Low	Medium	Low
Dissolution	High	Low	Medium	Medium	Low

FP CQAs: Finished product critical quality attributes, FB: Fluidized bed, Low: Broadly accepted risk. no further investigation is required, Medium: Risk is acceptable. further investigation may be needed in order to reduce the risk, High: Risk is unacceptable. further investigation is required to reduce the risk, overview of qualitative relative risk ranking system

Table 4: Quantitative failure mode effective analysis of critical process parameters affecting In process/finished product critical quality attributes

Unit Operations	Critical process parameter	Failure mode (critical event)	Effect on IP and FP CQAs with respect to QTPP (Justification of failure mode)	Severity (S)	Probability (P)	Detectability (D)	Risk priority No (RPN=S*P*D)
Fluid bed granulation	Liquid spraying rate	Higher RPM	Produce larger granules (lump)= Disintegration and dissolution can be affected	03	03	03	27
	Atomizing air pressure	Lower pressure	Unevenly distributes drug binder solution=Content uniformity can be affected	02	02	03	12
	Product temperature	Very high Inlet/Product/exhaust temperature	rate of degradation may be affected=Impurity profile may be affected	02	02	03	12
	Fluidizing air flow rate	Higher CFM	Attrition and evaporation produces fines by which process efficiency (%yield) can be impacted	03	03	01	09
Total RPN for FBP							60
Sizing	Sifting	Increase in sieve no.	Larger granules=dissolution may be affected	02	02	01	04
	Milling	Increase in Screen size	Uneven PSD=Content Uniformity can be affected	02	02	01	04
Total RPN for sizing							04
Blending	Blender RPM	Higher RPM	Increase No. of total	02	02	01	04
	Blending time	longer time	Revolutions=Disintegration and dissolution can be affected	02	02	01	04
Total RPN for blending							08
Compression	Press speed	High speed	Weight Variation~Content Uniformity may be affected, but in the case of FBP, individual particle coating gives very less deviation in CU	03	03	01	09
	Thickness adjustment	Higher hardness	Disintegration=Dissolution may be affected, but once range was set at pilot scale then there will be no effect at large scale	03	03	01	09
Total RPN for compression							18
Film coating	Bed temperature	Very high temperature	Impurity profile affected	02	02	03	12
	Spraying rate	Higher rate	Appearance affected	02	02	01	04
	atomizing air pressure	Lower pressure	Appearance affected	01	02	01	02
Total RPN for film-coating							18
Probability*	Severity**	Detectability***				Score	
Very Unlikely	Minor	High				01	
Remote	Major	Optimum				02	
Occasional	Critical	Low/No				03	

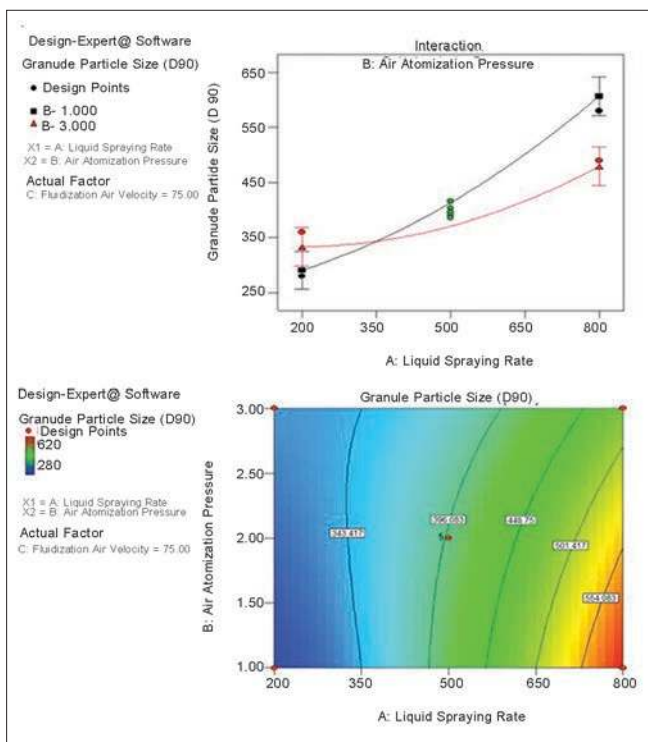Total risk priority number (RPN) more than 25 seek critical attention for DoE for possible failure
*Probability: likelihood of occurrence of harm to the quality, **Severity: Measure of the possible consequence of harm to the quality.,***Detect ability: Ability to discover or determine the existence, presence, or fact of harm to the quality.

Contour plots between one independent variable versus another holding magnitude of response and other variables constant of CPPs; with proven acceptable ranges and edges of failure were clearly revealed with defined margins in Figure 4. 3D Response Surface Plots (a graphical representation of a response variable plotted against two independent variables) and Cube plots (representing the effects of three factors at a time) were represented for both the responses in Figure 5.

Table 5: Box Behnken experimental design for quadratic model

Run	Factor 1 A: liquid spray rate (in g/min)	Factor 2 B: atomization air pressure (in bar)	Factor 3 C: fluidization air velocity (cfm)	Response 1 Y1: granule particle size (D50 in μm)	Response 2 Y2: process efficiency (in %)
1	2.00	2.00	100.00	336	87
2	2.00	2.00	50.00	320	92
3	2.00	1.00	75.00	280	92
4	2.00	3.00	75.00	360	95
5	5.00	3.00	50.00	380	96
6	5.00	3.00	100.00	360	89
7	5.00	2.00	75.00	396	97
8	5.00	1.00	50.00	460	97
9	5.00	2.00	75.00	416	98
10	5.00	1.00	100.00	440	93
11	5.00	2.00	75.00	404	98
12	5.00	2.00	75.00	386	96
13	5.00	2.00	75.00	390	97
14	8.00	1.00	75.00	580	97
15	8.00	2.00	50.00	620	96
16	8.00	3.00	75.00	490	98
17	8.00	2.00	100.00	530	95

Figure 4a: Interaction plot & 2D surface plot for the effect of Fluidized Bed Processing Factors (**A**: Liquid Spraying Rate & **B**: Atomization Air Pressure) on the Response Y1: Granule Particle Size (D90) (in um)

Figure 4b: Interaction plot & 2D surface plot for the effect of Fluidized Bed Processing Factors (A: Liquid Spraying Rate & D: Fluidization Air Velocity) for the Response Y2: Process Efficiency (in %)

Actual Equation for Response Y1: Granule size (D90) in Terms of Coded Factors:

Granule Size = +398.40 + 115.50 *A-21.25*

B-14.25*C-42.50*A*B-26.50*A*C + 0.000*B*

$$C + 35.30 *A^2 - 6.20*B^2 + 17.80* C^2 \quad (1)$$

The Model F-value of 24.82 implies the model is significant. There is only a 0.02% chance that a "Model F-Value" this large could occur due to noise.Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, AB, A^2 are significant model terms as mentioned in Table 6. Values greater than 0.1000 indicate the model terms are not significant. Here

there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve this model.

So, Reduced Equation for Response Y1: Granule size (D90) in Terms of Coded Factors:

Average Granule Size =+398.40 + 115.50

$$*A-21.25*B-42.50*A*B-+35.30 *A^2 \quad (2)$$

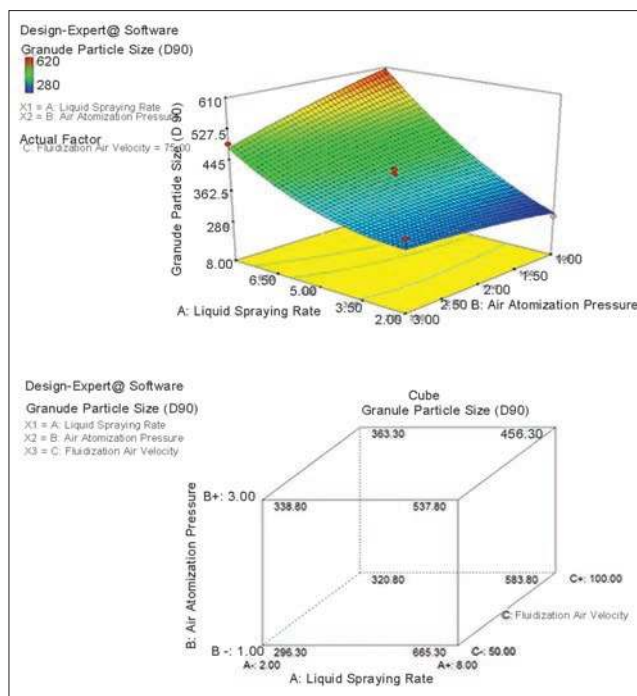Actual Equation for Response Y2: Process Efficiency (in %) in Terms of Coded Factors:

Figure 5a: 3D Surface plots & 4D Cube plots for FBP for the response Y1: Granule Particle Size (D90) (in um)
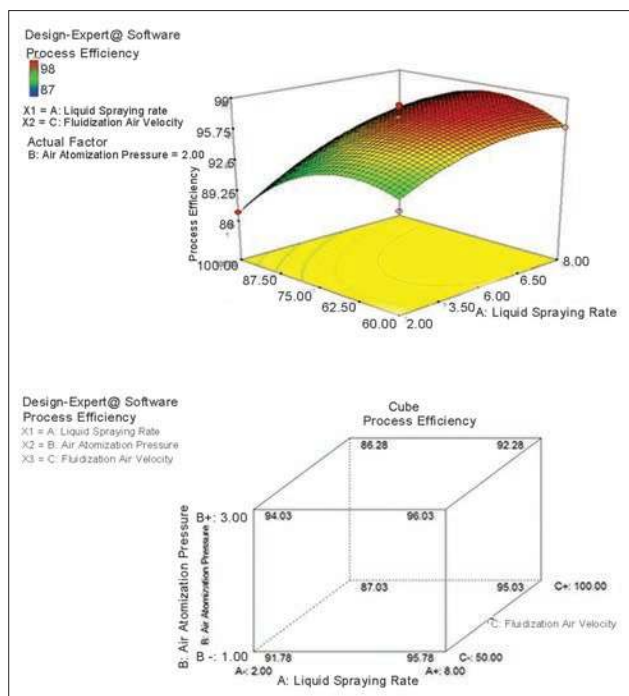

Figure 5b: 3D Surface plots & 4D Cube plots for FBP for the Response Y2: Process Efficiency (in %)

Table 6: Multivariate data analysis by ANOVA for response surface Quadratic model [Partial sum of squares- Type III] of response Y1: Granule size (D90) (in %) with effects from variables: A=Liquid spraying rate, B=Atomizing air pressure, C=Fluidization air velocity

Source	Sum of squares	Degree of freedom	Mean square	F value	P value prob>F	Model
Model	1.289E+005	9	14324.00	24.82	0.0002	Significant
A-Liquid spraying rate	1.067E+005	1	1.067E+005	184.91	<0.0001	
B-Air atomization pressure	3612.50	1	3612.50	6.26	0.0409	
C-Fluidization air velocity	1624.50	1	1624.50	2.81	0.1373	
AB	7225.00	1	7225.00	12.52	0.0095	
AC	2809.00	1	2809.00	4.87	0.0632	
BC	0.000	1	0.000	0.000	1.0000	
A^2	5246.69	1	5246.69	9.09	0.0195	
B^2	161.85	1	161.85	0.28	0.6128	
C^2	1334.06	1	1334.06	2.31	0.1722	
Residual	4040.20	7	577.17			
Lack of fit	3469.00	3	1156.33	8.10	0.0357	
Pure error	571.20	4	142.80			
Cor total	1.330E+005	16				

Table 7: Multivariate data analysis by ANOVA for response surface Quadratic model [Partial sum of squares- Type III] of response Y2: Process efficiency (in %) with effects from variables: A=Liquid spraying rate, B=Atomizing air pressure, C=Fluidization air velocity

Source	Sum of squares	Degree of freedom	Mean square	F value	P value prob>F	Model
Model	149.71	9	16.63	6.45	0.0112	Significant
A-Liquid spraying rate	50.00	1	50.00	19.39	0.0031	
B-Air atomization pressure	0.13	1	0.13	0.048	0.8320	
C-Fluidization air velocity	36.12	1	36.12	14.01	0.0072	
AB	1.00	1	1.00	0.39	0.5532	
AC	4.00	1	4.00	1.55	0.2530	
BC	2.25	1	2.25	0.87	0.3813	
A^2	9.16	1	9.16	3.55	0.1014	
B^2	0.21	1	0.21	0.083	0.7820	
C^2	43.79	1	43.79	16.98	0.0045	
Residual	18.05	7	2.58			
Lack of fit	15.25	3	5.08	7.26	0.0427	
Pure error	2.80	4	0.70			
Cor total	167.76	16				

Process Efficiency=+97.20 + 2.50 *A-0.12*B-2.13
*C-0.50 *A*B + 1.00*A*C-0.75*B
*C -1.47 *A^2-0.22*B^2-3.22*C^2   (3)

The Model F-value of 6.45 implies the model is significant. There
is only a 1.12% chance that a "Model F-Value" this large could
occur due to noise.Values of "Prob > F" less than 0.0500 indicate
model terms are significant. In this case A, C, C² are significant
model terms as mentioned in Table 7. Values greater than 0.1000
indicate the model terms are not significant. If there are many
insignificant model terms (not counting those required to support
hierarchy), model reduction may improve this model.

So, Reduced Equation for Response Y2: %Process Efficiency in
Terms of Coded Factors:

Process Efficiency= +97.20 + 2.50 *A-2.13*C-3.22*C^2   (4)

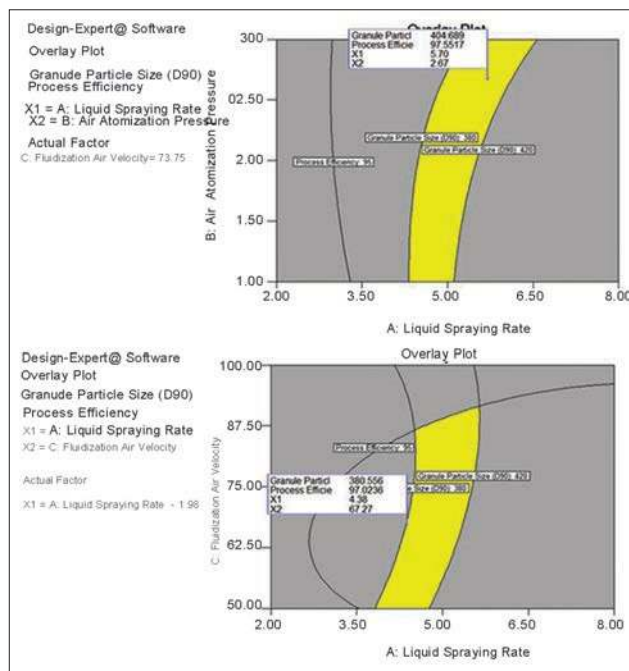**Figure 6:** Overlay plots of studied factors to achieve optimum
responses "sweet spots"

Table 8a: Constraints and 30 possible solutions of factors and responses

Factors: Process variables		Levels of factors studied		
		-1	0	+1
A	Liquid spraying rate (g/min)	2	5	8
B	Air atomization pressure (bar)	1	2	3
C	Fluidization air velocity (cfm)	50	75	100
Responses		Goal with acceptable ranges		
Y1	Granule particle size	To achieve D90 in the range from 380 to 420 µm		
Y2	Process efficiency	To achieve %yield in the range from 95% to 100%		

Table 8b: "Numerical Optimization" Constraints & 30 possible Solutions for combinations of Factors to achieve goal within acceptable ranges

Number	Liquid spraying rate (g/min)	Air atomization pressure (bar)	Fluidization air velocity (cfm)	Granule particle size (D90) (µm)	Process efficiency (%yield)	Desirability
1	4.25	1.47	56.08	391.695	96.0738	1.000
2	4.30	2.53	55.04	383.393	96.6143	1.000
3	4.89	1.64	84.86	397.973	95.8669	1.000
4	4.95	2.31	86.41	387.124	95.3498	1.000
5	5.05	1.36	86.75	408.7	95.7522	1.000
6	5.58	2.82	86.08	388.516	95.53	1.000
7	4.37	1.12	57.27	397.068	96.0253	1.000
8	6.32	2.75	86.57	414.493	95.8998	1.000
9	4.35	1.03	62.88	389.197	96.4216	1.000
10	5.71	2.69	88.72	397.219	95.1419	1.000
11	6.33	2.83	81.47	413.612	96.7656	1.000
12	5.97	2.85	64.00	418.739	97.897	1.000
13	5.66	2.97	70.99	393.513	97.5759	1.000
14	5.28	1.66	90.59	413.845	95.0911	1.000
15	4.45	1.10	64.03	393.106	96.638	1.000
16	5.05	1.21	67.71	419.807	97.3748	1.000
17	4.16	2.71	50.48	382.863	96.0596	1.000
18	4.74	1.26	62.68	408.688	96.9473	1.000
19	4.94	2.36	61.45	400.786	97.4424	1.000
20	5.52	2.82	81.28	388.197	96.4156	1.000
21	4.38	1.98	67.27	380.562	97.0238	1.000
22	5.38	2.56	65.98	405.543	97.7719	1.000
23	5.70	2.67	73.75	404.712	97.552	1.000
24	4.26	1.21	56.29	392.351	95.8764	1.000
25	5.41	1.78	89.66	416.059	95.3657	1.000
26	5.28	2.98	51.86	409.611	96.8323	1.000
27	5.28	1.40	85.72	418.594	96.1739	1.000
28	4.33	1.41	62.90	387.157	96.6604	1.000
29	5.38	1.64	87.76	418.047	95.809	1.000
30	4.97	2.91	59.25	388.958	97.3752	1.000

After analyzing experimental data by ANOVA and getting the final equation, the desired goal for each factor and response were chosen using "Numerical Optimization" option in Design Expert software. The goal seeking begins at a random starting point and proceeds up the steepest slope to a maximum as mentioned in Table 8a. By starting from several points in the design space chances improve for finding the "best" local maximum. The default is 30 starting points as mentioned in Table 8b.

With multiple responses, it was required to find regions where requirements simultaneously meet the critical properties, the "sweet spot". By superimposing or overlaying critical response contours on a contour plot, visually search for the best compromise was possible by "Graphical Optimization option" in Design Expert Software. Graphical optimization displayed the area of feasible response values in the factor space in yellow color. Regions that did not fit the optimization criteria were gray shaded as represented in Figure 6, constituted a DS for robust and rugged FBP.

Risk reduction by implementation of control strategy irrespective of scale

On the basis of overall development by QbD, a control strategy was designed to ensure that a product of required quality would be produced consistently by proposed process without probability of failure at larger scale. The elements of the control strategy described and justified how in-process controls and the controls of input materials (drug substance and excipients), intermediates (in-process materials), drug products container and closure system contributed to the final product quality. These controls were based on product, formulation and process understanding and include, at a minimum, control of the critical process parameters and material attributes. Sources of variability that impact product
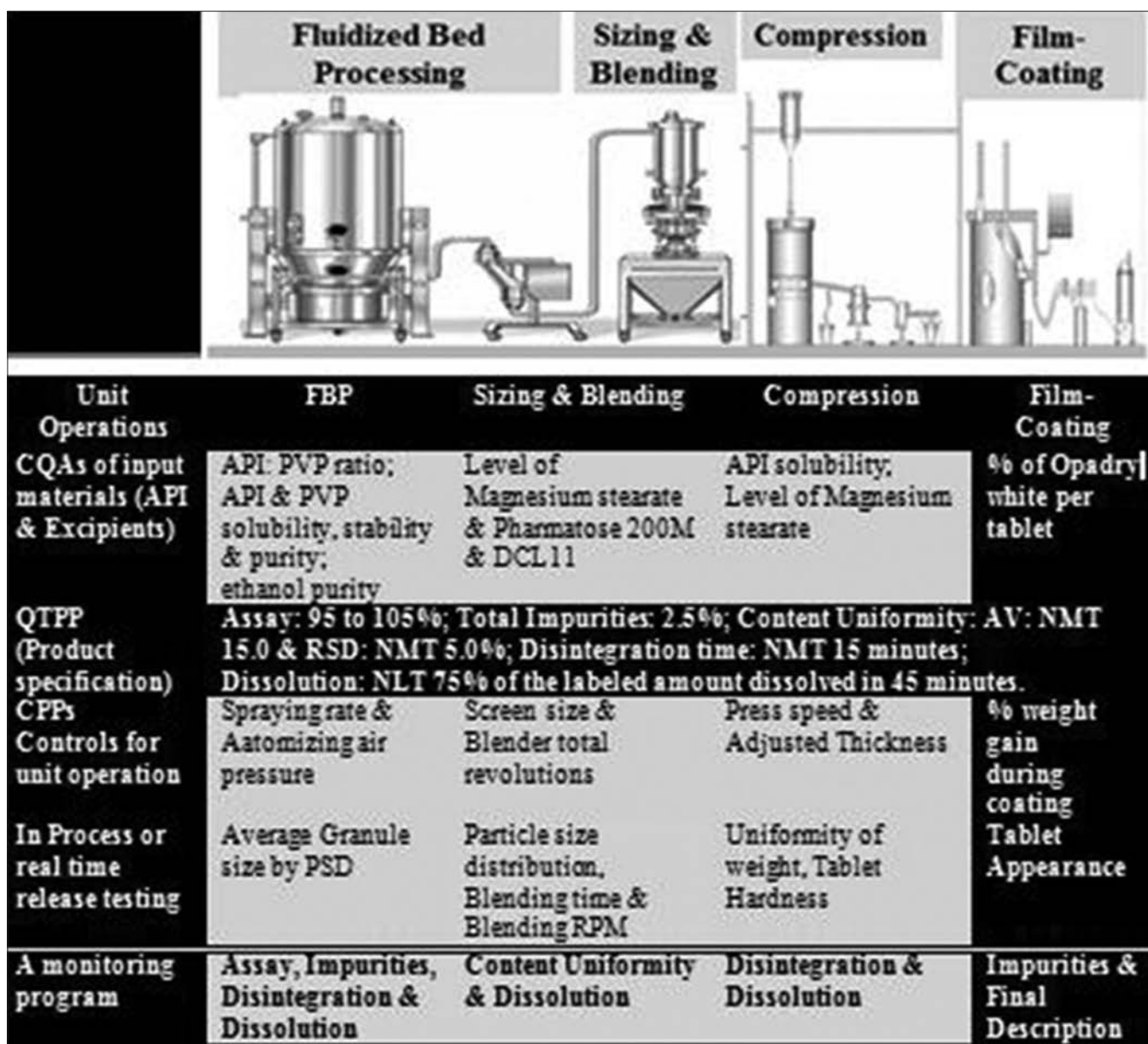

Unit Operations	FBP	Sizing & Blending	Compression	Film-Coating
CQAs of input materials (API & Excipients)	API: PVP ratio; API & PVP solubility, stability & purity; ethanol purity	Level of Magnesium stearate & Pharmatose 200M & DCL 11	API solubility; Level of Magnesium stearate	% of Opadry white per tablet
QTPP (Product specification)	Assay: 95 to 105%; Total Impurities: 2.5%; Content Uniformity: AV: NMT 15.0 & RSD: NMT 5.0%; Disintegration time: NMT 15 minutes; Dissolution: NLT 75% of the labeled amount dissolved in 45 minutes.			
CPPs Controls for unit operation	Spraying rate & Aatomizing air pressure	Screen size & Blender total revolutions	Press speed & Adjusted Thickness	% weight gain during coating
In Process or real time release testing	Average Granule size by PSD	Particle size distribution, Blending time & Blending RPM	Uniformity of weight, Tablet Hardness	Tablet Appearance
A monitoring program	Assay, Impurities, Disintegration & Dissolution	Content Uniformity & Dissolution	Disintegration & Dissolution	Impurities & Final Description

Figure 7: Outlined Controlled pertinent strategy for Robust & Rugged Manufacturing Process of Lacidipine Tablets

Figure 8: Controlled FBP Parameters from small lab scale to large production scale

quality were identified, appropriately understood and subsequently controlled. Understanding sources of variability and their impact on downstream processes or processing, in-process materials and drug product quality provided an opportunity to shift controls upstream and minimized the need for end product testing.

A final control strategy included the following as pointed out in Figure 7:

- Control of input material attributes especially risky formulation variables (e.g. drug substance, excipients, primary packaging materials) based on an understanding of their impact on process ability or product quality for reducing probability of risk;
- Product specification(s);
- Controls for unit operations especially risky process variables as per that have an impact on downstream processing or product quality (e.g. the impact of temperature on degradation, particle size distribution of the granulate on dissolution) for reducing probability of risk;
- In-process or real-time release testing in lieu of end-product testing (e.g. measurement and control of in process CQAs inline monitored by PAT tools: *in situ* Focused Beam Reflectance Measurement (FBRM) for inline Particle Size measurement during granulation and *in situ* Fourier Transform Infra-Red Spectroscopy (FTIR) for inline Blend Uniformity at Blending stage and Content Uniformity in Finished Product, which ensure that Fluidized Bed Granulation process is working as anticipated to deliver product quality attributes as predicted by the DS) for increasing detectability of harm;
- A monitoring program (e.g., full product testing at regular intervals) for verifying multivariate prediction models.

Pivotal scale-up considering prior pilot-scale QbD

Following section illustrated how a product was scaled up from 5 to 120 kg in equipment supplied by Glatt® when scaling up as revealed with "scale independent" fixed parameters in Figure 8. Scale-dependent variable parameters at large production (pivotal) scale were calculated as per following with reference to QbD optimized parameters at small laboratory (pilot) scale.

Batch size and equipment selection

Scale-up from small laboratory sized fluid-bed machines can be made much easier if the same line of equipment is to be used. However, efforts were in need to be spent on modifying process parameters, because of differences in air flow pattern, expansion chamber geometry, gun spray pattern, etc., Thus, for Top Spray equipment minimum and maximum batch size could be approximated as per equation no.(5) and (6)

$$Smin = [V \times 0.3 \times BD] = [500 \times 0.3 \times 0.4] = 60 \text{ kg} \qquad (5)$$

$$Smax = [V \times 0.7 \times BD] = [500 \times 0.7 \times 0.4] = 140 \text{ kg} \qquad (6)$$

Where; S is batch size in kilograms,
V is the product bowl working volume in liters
BD is the bulk density of finished granules in g/cc;
0.3 = Minimum occupancy of 30% in product bowl
0.7 = Maximum occupancy of 70% in product bowl

Fluidization air flow scale-up

To maintain the same fluidization velocity, the air volume in a larger unit was increased, based upon the cross-sectional area of the product bowl. In this case, the cross-sectional area of the base of the larger container was 0.64m^2 and the smaller was 0.02 m^2. Thus, correct air flow was calculated as per equation no. (7)

$$AF2 = [AF1 \times (A2/A1)] = [80 \times (0.64/0.02)] =$$
$$2560 \text{ CMH} \sim 2600 \text{ CMH} \qquad (7)$$

Where; AF_1 is Fluidization air flow in the laboratory scale equipment,
AF_2 Fluidization air flow in the scaled-up equipment,
A_1 is cross-sectional area of the laboratory scale equipment,
A_2 is cross-sectional area of the scaled-up equipment.

Spray rate and atomization air pressure scale-up

Spray rate scale-up was determined by the drying capacity of the equipment which is directly proportional to cross-sectional area of the air distribution plate rather than by the increase in batch size. At a given atomization pressure and air flow volume, change in liquid spray rate directly affects droplet size which in turn impacts particle agglomeration and may cause lumping. Thus, cross-sectional areas of the air distribution plate were used for approximation of scale-up spray rate as per equation no (8).

$$SR2 = [SR1 \times (A2/A1)] = [5 \times (0.64/0.02)] = 160 \text{ g/min.} \qquad (8)$$

Where; SR_1 is spray rate in the laboratory scale equipment,
SR_2 is spray rate in the scaled-up equipment,

Table 9: In process results for laboratory batch and scaled-up batch

IP CQAs	Laboratory batch results	Scaled up batch results
Appearance	White to off white free flowing granules	White to off white free flowing granules
Assay	98.6%	99.3%
Blend uniformity (*n*=11)	Mean: 99.4% Min: 96.6% Max: 102.2% RSD: 1.5%	Man: 101.5% Min: 97.1%% Max: 104.2%% RSD: 1.6
Average granule size	D50:360 µm	D50: 380 µm
Bulk density	0.45 g/cc	0.47 g/cc
Tapped density	0.54 g/cc	0.55 g/cc
Carr's index	16.67	14.54
Hausners ratio	1.20	1.17
Angle of repose	34˚	33˚
%Loss of drying	1.85%	1.72%

Table 10: Finished drug product results for laboratory batch and scaled-up

FP CQAs	Laboratory batch results	Scaled up batch results
Appearance	White to off-white, oval-shaped, coated tablets having embossed with "C" and "P" on one side with break line on both side.	White to off-white, oval-shaped, coated tablets having embossed with "C" and "P" on one side with break line on both side.
Assay	99.2%	99.7%
Impurities (Related Substances)	Impurity A: 0.31% Impurity B: 0.22% Any Other Impurity: 0.20% Total impurities: 0.72%	Impurity A: 0.55% Impurity B: 0.82% Any Other Impurity: 0.35% Total impurities: 1.72%
Content Uniformity (*n*=20)	Mean: 99.4% Min: 96.9% Max: 102.7% RSD: 1.4%	Mean: 100.9% Min: 97.7%% Max: 103.8%% RSD: 1.5%
Disintegration (*n*=6)	10 minutes	10 minutes
Dissolution (*n*=12)	98%	99%

FP CQAs: Finished product critical quality attributes

A_1 is cross-sectional area of the laboratory scale equipment, A_2 is cross-sectional area of the scaled up equipment.

To maintain the same particle size, the "triple-headed nozzle" in scale up could spray at the same pilot-unit spray rate at a same atomization air pressure. However, this could result in a longer process time. So another approach to maintain a similar droplet size was utilized to achieve granule size D90 of 400 µm with maintenance of the mass balance of spray rate and the atomization pressure by increasing the atomization pressure to 2*(3) =6 bar, the spray rate could be increased to 160* (3) =480 ∼ 500 grams per minute at production scale (where 3 indicates number of nozzle heads) keeping the same droplet size and hence obtaining granulation with desired CQAs as revealed in Table 9 and 10.

CONCLUSIONS

From exhaustive use of risk "assessment" tools: Qualitative Matrix Analysis and Quantitative Failure Mode Effective Analysis (based on Probability, Severity, and Detectability), it was unquestionable that Fluid Bed Granulation is the most critical step for achieving consistent QTPP in case of formulation of poorly soluble and highly biovariable drug LCDP by solid dispersion approach. To reduce/"control" risk irrespective of the scale, detailed experimental study of CPPs was carried out by BBD to develop DS with acceptable proven ranges, which reduce probability of risk respected CPPs affecting quality and/or performance of In Process/Finished Product (IP/FP) CQAs, which reduces probability of risk irrespective of scale. To increase detectability of risk, performance of FBP could be inline monitored by Process Analytical Technology (PAT) tools: *in situ* FBRM for inline particle size measurement during granulation and *in situ* FTIR for inline blend uniformity at blending stage and content uniformity in finished product, which ensure that fluidized bed granulation process is working as anticipated to deliver product quality attributes as predicted by the DS. Thus, understanding sources of variability of CPPs and "review" of impact of individual CPP on downstream processes or processing and finished product quality during pilot scale development stage provided flexibility for shifting of controls upstream at large scale and minimize the need for end-product testing and maximize the probability of process effectiveness at larger scale.

From the result of this QRM study of top spray FBP for antihypertensive drug formulation with Control Strategy engendered by "Box-Behnken Experimental Design Space", it has been proved that QRM, along with DoE and PAT tools of QbD can be a systematic process for the "assessment, control, communication, and review of any process-related risks to the quality of the drug product across the product lifecycle".

REFERENCES

1. Thiel WJ. The theory of fluidization and application to the industrial processing of pharmaceutical products. Int J Pharm Tech Prod Manuf 1981;2:5.

2. Schaefer T, Worts O. Control of fluidized bed Granulation IV: Effects of binder solution and atomization on granule size and size distribution. Arch Pharm Chem Sci Ed 1978;6:14-25.

3. Schaefer T, Worts O. Control of Fluidized bed granulation: II: Estimation of droplet size of atomized binder solution. Arch Pharm Chem Sci Ed 1977;5:178-93.

4. Schaefer T, Worts O. Control of fluidized granulation: III: Effects of the inlet air temperature and liquid flow rate on granule size and size distribution. Arch Pharm Chemi Sci Ed 1978;6:1-13.

5. Schaefer T, Worts O. Control of fluidized bed granulation, V: Factors affecting granule growth. Arch Pharm Chem Sci Ed 1978;6:69-82,

6. Schaefer T, Worts O. Control of fluidized bed granulation, I: Effects of spray angle, nozzle height and starting materials on granule size and size distribution. Arch Pharm Chem Sci Ed 1977;5:51-60.

7. Aulton MJ, Banks M. Fluidized bed granulation, factors influencing the quality of the product. Pharm Technol 1981;2:24.

8. Aulton MJ, Banks M. Fluidized bed granulation, factors influencing the quality of the product. Pharm Technol 1981;2:24.

9. Davies WL, Gloor WT Jr. Batch production of pharmaceutical granulation in fluidized bed I: Effects of process variables on physical properties of final granulation. J Pharm Sci 1971;60:1869-74.

10. Pellegatti M, Grossi P, Ayrton J, Evans GL, Harker AJ. Absorption, distribution and excretion of lacidipine: A dihydropyridine calcium antagonist, in rat and dog. Xenobiotica 1990;20:765-77.

11. Gannu R, Palem CR, Yamsani VV, Yamsani SK, Yamsani MR. Enhanced bioavailability of lacidipine via microemulsion based transdermal gels: Formulation optimization, *ex vivo* and *in vivo* characterization. Int J Pharm 2010;388:231-41.

12. Martinez MN, Amidon GL. A mechanistic approach to understanding the factors affecting drug absorption: A review of fundamentals, pharmacokinetics and pharmacodynamics. J Clin Pharmacol 2002;42:620-43

13. ICH (International Conference on Harmonization)-Q 8 (R2) Guideline for "Pharmaceutical development", Reference no: CHMP/ICH/167068/04, November 2005. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002872.pdf.[Last accessed on 2012 Dec 01]

14. ICH (International Conference on Harmonization)-Q 9 Guideline for "Quality risk management", Reference no: INS/GMP/79766/2011, February 2011, Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002873.pdf. [Last accessed on 2012 Dec 01]

15. ICH (International Conference on Harmonization)-Q 10 Note for guidance on "Pharmaceutical quality system", Reference no: INS/GMP/79818/2011, February 2011, Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002871.pdf. [Last accessed on 2012 Dec 01]

Author Help: Online submission of the manuscripts

Articles can be submitted online from http://www.journalonweb.com. For online submission, the articles should be prepared in two files (first page file and article file). Images should be submitted separately.

1) **First Page File:**
   Prepare the title page, covering letter, acknowledgement etc. using a word processor program. All information related to your identity should be included here. Use text/rtf/doc/pdf files. Do not zip the files.

2) **Article File:**
   The main text of the article, beginning with the Abstract to References (including tables) should be in this file. Do not include any information (such as acknowledgement, your names in page headers etc.) in this file. Use text/rtf/doc/pdf files. Do not zip the files. Limit the file size to 1024 kb. Do not incorporate images in the file. If file size is large, graphs can be submitted separately as images, without their being incorporated in the article file. This will reduce the size of the file.

3) **Images:**
   Submit good quality color images. Each image should be less than **4096 kb (4 MB)** in size. The size of the image can be reduced by decreasing the actual height and width of the images (keep up to about 6 inches and up to about 1800 x 1200 pixels). JPEG is the most suitable file format. The image quality should be good enough to judge the scientific value of the image. For the purpose of printing, always retain a good quality, high resolution image. This high resolution image should be sent to the editorial office at the time of sending a revised article.

4) **Legends:**
   Legends for the figures/images should be included at the end of the article file.