

**DETERMINATION OF THE EFFECT OF DIFFERENT BLADE SPEEDS AND
MIXING TIMES ON THE HOMOGENEITY OF MIXTURES CONTAINING
DIFFERENT RATIOS OF TWO POWDERS**

ELZAAN VAN WYK

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**A research report submitted to the Faculty of Health Sciences,
University of the Witwatersrand, Johannesburg, in partial fulfilment of the
requirements for the degree of
Master of Science in Medicine in Pharmaceutical Affairs**

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Johannesburg, 2014

DECLARATION

I, Elzaan van Wyk declare that this research report is my own work. It is being submitted for the degree of Master of Science in Medicine in Pharmaceutical Affairs at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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This ...22nd ...day of.....October....., 2014.

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.....*Evangelh*.....

This ...22nd ...day of.....October....., 2014.

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Father, Son and Holy Ghost for all the strength and guidance daily.

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ABSTRACT

DETERMINATION OF THE EFFECT OF DIFFERENT BLADE SPEEDS AND MIXING TIMES ON THE HOMOGENEITY OF MIXTURES CONTAINING DIFFERENT RATIOS OF TWO POWDERS

Aim

The first step in a wet granulation process is dry mixing. This step has the objective of ensuring that all the raw materials are mixed such that the end product is homogeneous. Dry mixing in a high shear mixer instead of a blender saves cost. However, the mixing parameters have not been well researched. Dry mixing parameters that are currently used, have been established through experience, trial and error and in-process testing. Alexander and Muzzio (2006) confirms this by stating that there are currently no mathematical techniques to predict blending behaviour of granular components without prior experimental work; therefore, blending studies start with a small-scale, try-it-and-see approach. Even though they are referring to blending, the same is also true for dry mixing. Both processes are the mixing of powders. Therefore the aim of this research was to develop parameters for dry mixing, based on experimental work.

Methods

Using a Saral rapid mixer and wet granulator (Saral Engineering Company, India), experiments were performed according to a 2^4 two-level Plackett-Burman Design method, to determine the effects of different blades (mixer/impeller and chopper) speeds and mixing times on the homogeneity of the mixtures containing different ratios of two powders that have different densities and particle sizes. One of the powders mixed, was enalapril maleate. This was chosen as it can be assayed. Samples were taken from the bowl and tested for assay. The mix for a specific experiment is homogeneous if the results of all 7 assayed samples are within 10 % of the target % w/w value and the % Relative Standard Deviation (% RSD) of the 7 results is less than or equal to 5,0 %. The outcome was being measured in % RSD. A lower % RSD indicates a more homogeneous mix.

The parameters developed, will be beneficial to pharmaceutical companies as it can assist them to improve accuracy, consistency and quality of granular mixes. The experimental method used can serve as an example for future experiments.

Results

The results indicated that impeller blade mixing speed and mixing time are the two factors that have the biggest impact on the homogeneity of a mix in a high shear mixer. Chopper blade speed was also found to be significant, but less than the above two parameters mentioned. Optimal parameters were predicted.

Conclusion

As there are many parameters to be controlled during dry mixing in a high shear mixer, a statistical design method is suitable to establish the parameters that would have the most impact on the end result. Statistically it was found that mixing speed of the main impeller and chopper blades and overall mixing time are the three factors that have the biggest impact on the homogeneity of a mixture. The mixing time and impeller blade speed have proven to be more significant than the chopper blade speed. Concentration was found to be insignificant. For our experiments and for the specific granulator used the following optimal parameters could be deduced: Impeller blade set at 191 rpm, chopper blade set at 2002 rpm and mixing time set at 3.01 minutes.

Keywords: wet granulator, dry mixing parameters, blade speeds, 2^4 two-level Plackett-Burman Design method

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LIST OF ABBREVIATIONS

% RSD	Percentage Relative Standard Deviation
% w/w	% weight/weight
API	Active Pharmaceutical Ingredient
CFR	Code of Federal Regulations
DoE	Design of Experiments
FDA	Food and Drug Administration
IPI	Inactive Pharmaceutical Ingredient
MCC	Medicines Control Council
MHRA	Medicines and Healthcare products Regulatory Agency
NIR	Near Infrared
Rpm	Revolutions per minute
R&D	Research & Development
RSM	Response Surface Methodology
USFDA	United States Food and Drug Administration
USP	United States Pharmacopoeia

1. INTRODUCTION

1.1 Background to research

Tabletting entails compressing powders or a granule into a tablet. Encapsulation entails filling a capsule shell with powder or granules. Dry mixing is part of any granulation process. Dry mixing needs to be well understood, as well as all the factors influencing it. This mixing process has a critical impact on the content uniformity of the final product, whether a granule from a wet granulation process, dry powder for direct compression or roller compaction, or a dry powder for encapsulation. The purpose of dry mixing of powders before a wet granulation process is to get an even distribution of all the ingredients in the mixture, including the Active Pharmaceutical Ingredient (API) raw material in the powder mix, before adding the granulating medium to form a coherent and homogenous granule. This is to ensure that the final granules, which are compressed into tablets, contain the same amount of the API, so that the patient consistently consumes the required dose of API. It is also to ensure that all the Inactive Pharmaceutical Ingredients (IPI's) are contained in the same amounts for the tableting procedure. Each ingredient in a mix has a specific purpose and the correct quantity should be in each tablet, for example if the disintegrant is not homogeneously mixed, then the tablets might disintegrate at different rates, which could lead to different dissolution times and finally different levels of the medicine in the blood. The dry mixing of powders is an important operational step, because the accuracy of the API content of the tablets depends upon the efficiency of mixing of the dry powders before the wet granulation process takes place. Content uniformity of the final dosage form is dependent on the homogeneity of the powder mixture in the mixer/ blender. Alexander and Muzzio (2006) confirms this when stating that in the manufacture of many pharmaceutical products (especially tablets and capsules), dry particle blending is often a critical step that has a direct impact on content uniformity. This statement is further confirmed by Venables & Wells (2001) who state that mixing is the fundamental process in solid particulate dosage forms to ensure content uniformity and, in many cases, dissolution rate.

Dry powders could be mixed manually in a trough, it could be mixed in a high shear mixer or it could be mixed in a blender before it is granulated. A satisfactory mixing process should produce a uniform mixture in minimum time and with minimum cost for overhead, power and labour (Barbosa-Cánovas et al., 2005). Wet granulation is used extensively in pharmaceutical industries. The popularity of wet

granulation is because it can be applied to all drugs, and for many formulators it is the method of choice for drugs with high doses and a very low dose (DFE Pharma 2013). In the pharmaceutical industry, most products are manufactured using the wet granulation process. More than 70 % of the global industry's granulators are made using this method (Tousey 2002). Vojnovic et al. (1992) confirm the extensive use of wet granulation in their article titled "Wet Granulation in a small scale high shear mixer". The three commonly used granulation methods include wet granulation, dry granulation, and hot-melt granulation (Gokhale & Trivedi 2010). Currently, particle blending is a key step in most industrial processes, especially in the manufacture of pharmaceuticals, where 80 % of drug products are sold as solid dosage forms (tablets and capsules) and subject to stringent FDA regulations on content uniformity (Sudah et al., 2002).

The different stages of wet granulation includes:

Dry mixing – wet granulation – drying – screening – blending

By performing the dry mixing step in the same equipment in which the product is granulated, will save time in terms of equipment and operator labour. To use a blending system first for mixing and then discharge into a bowl of a high shear mixer/granulator to form granule, is more costly and time consuming than to perform the dry mixing and granulation steps in the same equipment. Numerous studies have been performed on dry mixing in blenders (for example: Bozzone 2001 and Ngai 2005), but very few have been performed on dry mixing in a high shear mixer/granulator. Since high shear mixers and blenders use different types of mixing (diffusion or convection versus shear), parameters are different and studies performed on one cannot be applied to the other equipment. Parameters in diffusion mixing would include the speed that the blender bin rotates. Diffusion mixing entails mobility of individual particles being increased. Particles are distributed over a freshly developed interface. Parameters in convection mixing would include the rotating speed of a paddle for example. Convection mixing entails a random motion of solid particles. Groups of particles are moved from one position in a mixer to another due to the cascading of materials within the equipment. Parameters in shear mixing include the speed that the impeller/mixer and chopper blades rotate. Shear mixing entails the development of slip planes within a bed of materials or the splitting of the bed of material to disintegrate agglomerates to overcome cohesion. In a shear mixer it is the impeller and chopper blades that rotate, whereas in diffusion mixing it is the bin itself and in convection mixing it is one

paddle or impeller that rotates. Principles that influence the mixing of powders can be applicable to both mixing systems, but the operating parameters will be different as a high shear mixer has blades which are not present in a blender. The reason for very little available literature on dry mixing parameters in high shear mixers might be because this step forms part of the development of a granule and is tested as an in-process step which becomes confidential information for a company. It is a test that is done as part of experimentation and not as part of routine in-process testing. It is also because the parameters might be specific for each and every product. This project is an example of how optimal parameters for dry mixing in a high shear mixer can be established during the development phase, especially for a product where content uniformity is a challenge. Small tablets or tablets that has a low mass as well as a small percentage of API (low dose) in the tablets pose a challenge to content uniformity. An example would be enalapril tablets which have a weight of for example ± 150 mg and a strength of 20 mg. Less than 15 % of the tablet mass is API.

The position of the US Food and Drug Administration (US FDA) on blend testing is as follows: when mixing is critical, blend evaluation is warranted, but may be unnecessary under certain circumstances (FDA 2003). It is hence not compulsory to test a dry mix before wet granulation, but only after the blending step, which is when the granule and extra-granular material have been mixed together.

Code of Federal Regulations (CFR) Title 21 (21 CFR 211.110) requires in-process controls and tests to monitor those steps responsible for variability in the process of manufacturing. One of these in-process control tests is a test for the adequacy of mixing to assure uniformity and homogeneity.

“ (a) To assure batch uniformity and integrity of drug products, written procedures shall be established and followed that describe the in-process controls, and tests, or examinations to be conducted on appropriate samples of in-process materials of each batch. Such control procedures shall be established to monitor the output and to validate the performance of those manufacturing processes that may be responsible for causing variability in the characteristics of in-process material and the drug product. Such control procedures shall include, but are not limited to, the following, where appropriate:

(3) Adequacy of mixing to assure uniformity and homogeneity” (US FDA 2013).

Validation may be defined where it is appropriate, but a conclusion cannot be made before validation is completed and historical data is analysed. Testing for adequacy of mixing is performed after blending and content uniformity is tested after compression into tablets, but testing of adequacy of mixing may be warranted after dry mixing for a product that has challenges with passing these tests. In this code it is specified that in-process specifications shall be consistent with drug product final specifications. The limits applied to the testing of content uniformity after blending might be applied to the testing after the dry mixing step.

Testing after dry mixing is one way to assure adequacy of mixing and hence uniformity and homogeneity. The final objective of any powder mixing process is to produce a homogenous blend of all powders in the mix, but even determining mixture composition throughout the dry mixing of powders is a difficulty.

In January 2011, the FDA released its first major process validation guidance in more than twenty years (US Department of Health and Human Services 2011). With this guidance came the introduction of a lot of new terms: eg. Design Qualification, Process Development requirements, ongoing Process Verification monitoring, Quality by Design, Critical Quality Attribute and the term Design Space. Process Validation is now performed across the entire product lifecycle. During the first stage, which would be Process Design, companies will need to document the processes performed during the development phase of a product. Studies need to be conducted to identify and control sources of variability. This is the stage where process knowledge and understanding needs to be built and captured. When the effects of factors on a pharmaceutical process or response are unknown, the use of screening designs to estimate the factor effects may be important to be used.

According to the FDA directive (FDA 2011), manufacturers of pharmaceutical products, should:

- Understand the sources of variation in the process of manufacturing a medicine
- Detect the presence and degree of variation
- Understand the impact of the variation on the process and ultimately on product attributes
- Control the variation in a manner commensurate with the risk it represents to the process and product.

The unit operation in which two or more materials are interspersed in space with one another is one of the oldest and yet one of the least understood unit operations in process engineering (Barbosa-Cánovas et al., 2005). The mixing of particles in the dry solid state is one of the oldest industrial processes known to man (Sudah et al., 2002). In the agricultural, food processing and the pharmaceutical industry, mixing operations are used to blend ingredients. The importance of this processing step is emphasized in the article by Swaminathan: Solid mixing is an important unit operation in the manufacture of pharmaceutical oral solid dosage forms. The importance of producing stable mixtures to ensure the uniformity of dosage units with respect to the active ingredient, particularly low-dose potent active ingredients, cannot be overstated. Whereas the uniformity of a blend does not in itself guarantee uniformity of the drug in a final dosage form, producing a stable mixture still remains the first important step in solid dosage form manufacture (Swaminathan & Kildsig 2002). Dry mixing parameters in a high shear mixer/granulator that are currently used have been established through experience, trial and error and in-process testing by means of measuring the active drug after blending. Note that it states: assay method after blending and not after dry mixing. Data on dry mixing parameters used in high shear mixers/granulators in pharmaceutical companies is confidential information; therefore they are not freely available or published.

Ensuring homogenous mixtures of APIs and excipients and avoiding segregation (or de-mixing) of these mixtures are challenges that pharmaceutical companies face everyday. Despite its importance, no definitive and robust approach has been established to quantitatively describe blending dynamic (Ngai 2005). Bozzone (2001) suggested that future needs and trends have been identified, one of which is to study mixing in equipment with different principles such as high shear mixing. He recommended that when blending is shown to be a critical process step, an adequate pilot study of the causative factors during process optimisation should be ensured. If a blend fails the assay test, it is important to go back and ensure that the initial dry mixing step was adequate for mixing the powders. Previous investigations on mixing of cohesive powders, in general, and pharmaceutical materials, in particular, have been limited (Sudah et al., 2002).

The pharmaceutical company where the Master's project was performed has had questions from MHRA (Medicines and Healthcare products Regulatory Agency) auditors from the United Kingdom, asking how they established the parameters for dry mixing of the powders in the high shear

mixer/granulator. The question posed were about how it is known that the mixing at the dry mixing stage is adequate if not tested? The FDA also wants experiments performed by companies to be documented. They require manufacturers to use Design of Experiments to try and establish where variability in the process lies. Plackett-Burman is one example of Design of Experiments that can be used in the pharmaceutical industry. Quality by Design level knowledge of the critical control parameters for key functional performance of a dosage form prior to routine manufacture and subsequent validation is becoming more important in the pharmaceutical industry.

The predicted outcome of this study is to assess the impact of blades speeds and mixing time on mixtures of three different ratios of two powders with different densities and particle sizes. This study will either discover new optimal parameters or confirm current parameters used on this equipment. It will be valuable for the pharmaceutical company in that substantial evidence will be available (together with current available data) for optimal dry mixing parameters. This could provide answers to possible questions during future audits by regulatory bodies. Proven parameters, based on thorough research, can also improve consistency and improve product quality and provide confidence to the company that the data they provide to the auditors would be acceptable. Since there has been very little published research on dry mixing parameters together with the requirements of the new validation guidelines as well as a question posed during an audit, research on dry mixing parameters is necessary and hence this research intends to establish optimal mixing parameters by using Experimental Design.

1.2 Statement of problem

The pharmaceutical manufacturer at which these studies were carried out have purchased a granulator (Saral Engineering, India) which they use for mixing of powders and for wet granulation during the development phase of products. The high shear mixer has an impeller and chopper blades which can be set to achieve effective mixing of pharmaceutical powders. In order to carry out the mixing process, one needs to determine the ideal settings to mix a typical sample of powder mix effectively. Design of experiments could be used to assist.

Specific products might fail assay testing after the blending step, in which case it is important to ensure that the dry mixing parameters were adequate to ensure adequacy of mixing.

As mentioned earlier: Bozzone (2001), Ngai (2005) and Sudah et al. (2002) investigated blending in blenders. The data generated from these studies is for mixing in blenders and not high shear mixers/granulators.

1.3 Aim of the study

The aim of the study is to determine the optimal settings (speed of impeller and chopper mixing blades and mixing times) for dry mixing of typical pharmaceutical powders during the manufacture of a product in a high shear mixer/granulator. Three different ratios of two powders (enalapril maleate and lactose monohydrate) of different densities and particle sizes will be used. One of the powders' content is quantifiable.

1.4 Objectives

The study objectives are:

- To determine how well the mixer mixes three different ratios of two powders with different densities and particle sizes at three different impeller blade and chopper blade speeds and for three different time periods.
- To determine the optimal parameters for dry mixing using fractional factorial design.

1.5 Overview of the research

Chapter 1 briefly describes the background to the research as well as states the aim and objectives. This chapter summarizes blending and mixing and explains the need for experimentation on dry

mixing parameters in a wet granulation process. The statistical method used in this project is a Plackett-Burman design.

Chapter 2 is a brief outline of the research that has been done on dry mixing and the parameters and describes the important theoretical considerations. The research performed using this statistical method in the pharmaceutical industry is discussed. This chapter describes the experiments to be done in this project.

Chapter 3 covers the methodology of the research. This chapter elucidates the materials used and methods applied in this study.

Chapter 4 outlines the results, and the discussion thereof follows in chapter 5.

The recommendations are contained in chapter 5.

2. LITERATURE REVIEW

2.1 Mixing of Pharmaceutical powders

Although scarce, work focusing on the mixing of pharmaceutical formulations dates back to 1962, when Kaufman mixed penicillin G and dihydro-streptomycin sulfate in a double cone and V tumbling blenders at various fill levels. One of his observations was that active concentration did not affect the homogeneity of a mixture. The statistics were based on 10 samples per experiment obtained using thief samplers (Kaufman 1962).

Ngai (2005) explains that there are many factors influencing mixing. These include: fundamental parameters intrinsic to the powder particles, such as size, geometry, moisture content and chargeability. These intrinsic fundamental parameters have an influence on the primary effects of the powders, including physical interlocking, liquid bridging and tribo-electrostatic interactions. These primary effects will ultimately have an effect on secondary effects such as cohesive and frictional forces between particles. These secondary effects together with the operating parameters then determines the blending kinetics, which includes particle motion, aggregation, deformation and breakup (Ngai 2005). Apart from the properties already mentioned, surface properties, flow characteristics, friability, moisture content, and tendency to cluster or agglomerate, may influence the tendency to segregate. The closer the ingredients are in size, shape, and density, the easier the mixing operation and the greater the intimacy of the final mix (Barbosa-Cánovas et al., 2005).

The operating parameters include three factors: environmental factors such as humidity and temperature; mechanical factors including blend geometry, blender size and blender material as well as system factors including initial powder fill volume and blender rotation rate (which in this project relates to *mixer blade speeds*) (Ngai 2005). For this experiment humidity, temperature, mixer geometry, mixer size, mixer material and fill volume will stay constant. The choice of an appropriate mixer along with *mixing time* is important (Harnby 1967). The same mixer will be used for all the experiments. Venables and Wells (2001) confirm the above when stating that particle size, particle shape, particle density, particle charge, choice of mixer, *mixing time* and *drug concentration* are factors affecting blend uniformity. Barbosa-Cánovas et al. (2005) state that powder blending is mainly affected by the *mixing time*, the design of the mixer (including size, shape, paddle geometry, and

rotational speed) and type of powders being mixed. Drug content variation increases with decreasing drug content, and this is therefore important when attempting to achieve blend uniformity with a low-dose drug (Egermann & Pichler 1988 cited in Venables & Wells 2001). Drug:excipient ratio has no effect (Venables & Wells 2001). Although the small concentration of active ingredient in a low-dose formulation (<1% w/w) has always been assumed to be the reason for poor content uniformity, not much experimental evidence is available (Venables & Wells 2001). A study performed by Greaves et al. concluded that content uniformity is improved when the active ingredient is dispersed in an aqueous system. This was then used as the granulating agent in the wet granulation process (Greaves et al., 1995). Using this approach would hence exclude the testing of the dry mix, as was performed in this project. A small amount of cohesive active could be pre-blended with a much larger amount of free-flowing excipient. This could also aid with content uniformity (Alexander et al., 2004). The challenge of content uniformity when there is a small concentration of active in the mix is one that could be overcome. Alexander and Muzzio (2006) that discuss dry blending and mixing in blenders confirm the variable parameters in mixing when discussing scaling up for dry Blending and Mixing: by stating that the questions that arise include: What rotation rate should be used?; How long should the blender be operated?. They also confirm that there is no generally accepted method for approaching answers to these questions; and therefore ad hoc approaches tend to be the rule rather than the exception.

Three mechanisms have been recognised in solids mixing: convection, diffusion and shear (Barbosa-Cánovas et al., 2005). In any particular process one or more of these three basic mechanisms may be responsible for the course of the operation. High shear mixers mostly have shear mixing.

Shear mixing is induced by the momentum exchange of powder particles having different velocities (differential velocity distribution). Shear mixing is developed by the formation of slipping planes in the bulk material; the originally coherent particle groups are gradually broken along these planes. The velocity distribution develops around the agitating impeller and the vessel walls due to compression and extension of bulk powders (Barbosa-Cánovas et al., 2005). It is important to note that the operating principle in blenders differ to those in high shear mixers. The literature on studies performed in blenders are mostly in blenders that uses diffusion mixing. Due to the difference in operating principles, the results from these studies cannot be applied directly to high shear mixers/ granulators.

In a high shear mixer, blades are used to mechanically dilate and shear the powders. Hickey and Ganderton (2001) state in their book on Pharmaceutical Process Engineering that “since the shearing of particles in a bed to achieve a uniform mix or blend is a statistical process, it must be monitored for efficiency. Sample thieves are employed to probe the powder bed, with minimal disturbance, and draw samples for analysis. These samples are then analyzed for the relevant dimension for mixing, eg. drug content. Statistical mixing parameters have been derived based on mean and standard deviation of samples taken from various locations in a blend at various times during the processing”.

In any operation mixing and de-mixing may occur concurrently, and the intimacy of the resulting mix depends on the predominance of the former mechanism over the latter. Once the mixing and de-mixing mechanism reach a state of equilibrium, the condition of the final mix is determined and further mixing will not produce a better result (Barbosa-Cánovas et al., 2005). A chopper blade is used to create the necessary shear forces to break up agglomerates of cohesive materials.

A study performed by Samuel Ngai (2005) on blenders showed that the time required to reach a homogenous mixture increased with blender fill volume and decreased with blender rotation rate (rpm). With respect to the effect of operating parameters on blending kinetics, the simulations showed that the homogenous mix increases as fill volume increased, rpm decreased, or when Microcrystalline Cellulose as opposed to lactose was chosen as an excipient. The experiments were performed in lab blenders. Even though dry mixing and blending is supposed to have the same function, to create an evenness of the mixture, blenders operate by convection and diffusion mixing and it is hence very difficult to apply conclusions made from studies performed on such equipment to the mixing in high shear mixers/granulators. A guideline for mixing was drawn up by Venables and Wells (2001) and four of the factors mentioned include: Drug concentration (low drug concentration can lead to poor content uniformity), Mixer choice (use segregating mixers for friable or unisize particles and use nonsegregating mixers for materials prone to segregation), Mixing time (must be optimized for each mix to minimize segregation) and Sampling (use common sense to determine a suitable sample size, sample the whole stream for many short periods of time and sample while the powder is in motion). From the study performed by Sudah et al. in blenders (2002), it was also concluded that the concentration of the active component did not affect the outcome of the mixing process, formulations at 3 % and 30 % KCl generated nearly identical results. Extensive work has been performed for active

distribution in blending systems, but data on mixing/blending in a high shear mixer system is minimal. Since it is easier to perform tests on lab-scale equipment that matches production, the burden is on development organisations to obtain appropriate high shear mixers based on proper scaling down of those that will be in production.

Vojnovic et al. (1992) state that “wet granulation is a technological process of size enlargement used in the pharmaceutical industry to prepare powdered materials for capsules and tablets. Despite the extensive use of the technique there is a lack of systematic research concerning the relationship between process variables and granule properties on the small scale high shear mixers”. Dry mixing is the first step in wet granulation.

Powder mixing is a complex process. Even today, there is no simple list of rules that can be followed to create a perfect mix; however, there are guidelines that can be followed to minimize segregation and agglomeration (as mentioned previously from research performed by Venables & Wells). By continually updating mixing methods used in the pharmaceutical industry, these problems can be removed, and powder mixing can be made a much more efficient process (Venables & Wells 2001). Venables & Wells (2001) state that their article on powder mixing is part of a much larger subject for which far more work is needed. Not enough time is taken by most pharmaceutical companies to raise awareness of the bias and other errors when mixing and sampling, its importance is underestimated (Venables & Wells 2001).

With the new Validation guideline from FDA in 2011 it has been made clear that more statistics will need to be used to prove that the process is capable of consistently delivering quality products. In the introduction of this guideline it states: “The guidance aligns process validation activities with a product lifecycle concept and with existing FDA guidance, including the FDA/International Conference on Harmonisation (ICH) guidances for industry, *Q8(R2) Pharmaceutical Development*, *Q9 Quality Risk Management*, and *Q10 Pharmaceutical Quality Systems*. Although this guidance does not repeat the concepts and principles explained in those guidances, FDA encourages the use of modern pharmaceutical development concept, quality risk management, and quality systems at all stages of the manufacturing process lifecycle” (FDA, 2011). In the International Conference on Harmonisation Harmonised Tripartite Guideline (Q8(R2) Pharmaceutical Development) it also specifies that critical process parameters are generally identified through an assessment of the extent to which their

variation can have an impact on the quality of the drug product (ICH 2009). This project can serve as an example to industry of how to incorporate the guidelines and Design of Experiments. One of the recent developments in the validation of pharmaceuticals is risk assessment. In figure 1, a fishbone diagram is used to identify the potential variables which can have an impact on the desired quality attribute, which in this project is the homogeneity of the mixture.

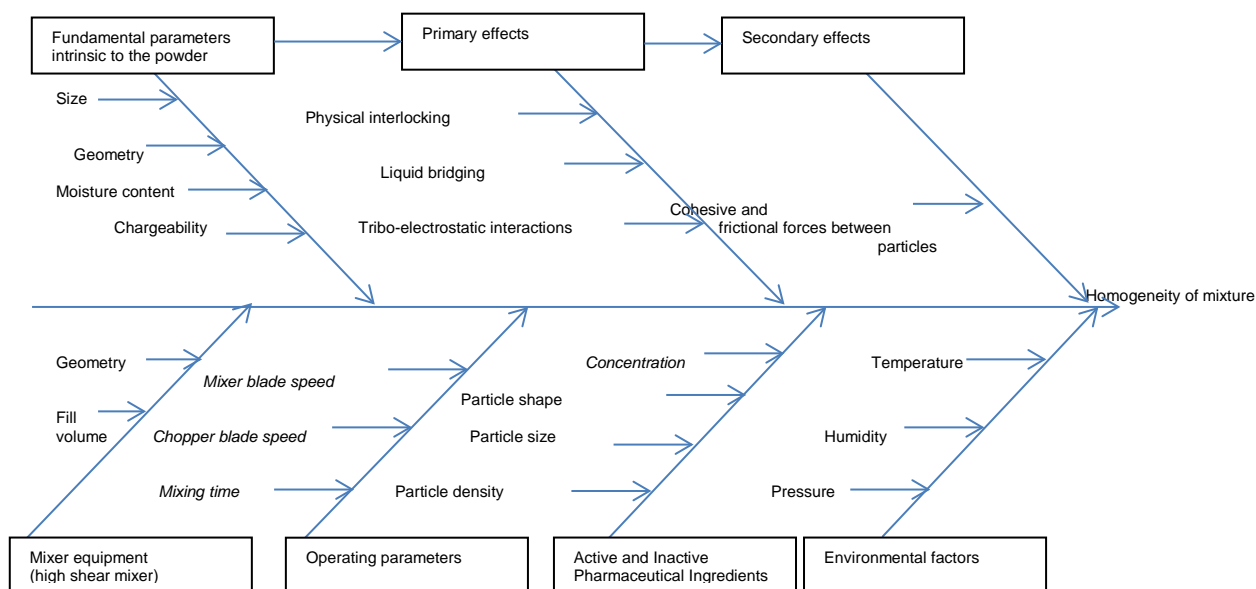


Figure 2.1: Fishbone diagram for risk analysis

Four potential variables have been chosen from the knowledge gained from literature review and from probability, severity and detectability of the variables based on prior knowledge which can have an impact on the quality attribute. Experimental data will be created in this project that can be used to make decisions in future. Design of experiments is used to evaluate the impact of the higher ranked variables, to gain greater understanding of the process and to develop a proper control strategy to remain within the design space established for a certain product. This is data that can be shown to auditors and can form part of the initial development process.

2.2 Statistical optimisation

Experimentation is expensive in terms of time, work force, and resources. It is therefore practical to ask whether experimentation can be made more efficient, and hence reducing expenditure of time and money (Armstrong 2006). A study on wet granulation in a small scale high shear mixer used

experimental design. There were three process variables (moisture level, impeller blade speed and granulation time). The conclusion drawn from this study was that centered composite design may be used in the pharmaceutical process of granulation to plan the experimental design and to find the main effects and interactions of the process variables on the physical properties of the granulate. The method is also recommended to optimize the process variable in a laboratory scale high shear mixer (Vojnovic et al., 1992). Another study assessing the influence of chopper blade and mixer/impeller blade speeds and microwave power level during the high-shear granulation process on final granule characteristics successfully used central composite factorial design (Kiekens et al., 1999).

Operating parameters are often chosen according to previous experience, by expert knowledge or by systematic screening. This project concentrates on statistical experimental design-based optimization. Design of experiments can be used to optimize an operating process. Design of experiments is used in this project. Plackett-Burman design can be used to establish out of many variables that might affect the outcome of an experiment, which one will prove the most important and hence justify more extensive study. Venables & Wells (2001) conclude in their article that: "When analysing the variance of a mix, many methods are available. Indices still remain popular today, as does the Poisson distribution, but both methods have their limits, and the ANOVA technique investigated by Rollins et al. may provide a useful alternative, especially for segregation determination". Plackett-Burman design was chosen for this project as it is a fractional factorial design. A fractional design requires a limited number of experiments. This is desirable for industry as experiments takes time and is costly. The type of design is hence a factorial design type that can be used again in this industry. It is anticipated that the type of design will provide the information that is required from the experiments- to determine optimal parameters.

Singh (2011) states in his article that formulation development of the oral drug delivery systems cannot be adequately accomplished using the traditional 'trial and error' approaches of one variable at a time. Ngai (2005) also confirms this by stating that due to a lack of fundamental understanding in powder mixing makes trial and error the best practice in the industry. This calls for the adoption of rational, systematized, efficient, and cost efficient strategies using 'design of experiments'. He also says that the recent regulatory guidelines issued by the key federal agencies to practice 'quality by design' paradigms have coerced researchers in industrial milieu, in particular, to use experimental

designs during the product development. This would include design of experiments (DoE), of which Plackett-Burman would be an example of a DoE that can be used for screening. Design of experiments is also a tool that can be used during risk evaluation. A design space could be created for a specific product based on DoE. This can aid in risk decision making. This use of experimentation is also mentioned in the article by Charoo & Ali: "The objective of ICH Q8, Q9 and Q10 documents is the application of systemic and science based approach to formulation development for building quality into product. There is always some uncertainty in new product development. Good risk management practice is essential for success of new product development in decreasing this uncertainty" (Charoo & Ali 2013).

Currently no mathematical techniques exist to predict blending behaviour of granular components without prior experimental work. Therefore, blending studies start with a small-scale, try-it-and-see approach (Alexander & Muzzio 2006). Actual knowledge in mixing is a combination of "know-how" and science, in which "know-how" is predominant and only a few individuals and specialized companies around the world have a thorough knowledge of this matter (Barbosa-Cánovas et al., 2005). Most of the powder mixing science in academia and industry is based on empirical approaches or experience based correlations, allowing only limited understanding of the blending behaviours of pharmaceutical powders (Crowder & Hickey 2000 cited in Ngai 2005). Ngai states that this lack of fundamental understanding in powder mixing makes trial-and-error the best practice in industry. Blend samples analysis should be conducted on development batches by extensive testing in the mixer. Advances in quantitative measures of dry solids mixing help to control mixer performance. In actual practice, however, a mixer is tested by the properties in the mixed material that it produces. Developments in mathematical modeling of mixing processes are scarce and established procedures for process design and scale-up are lacking (Barbosa-Cánovas et al., 2005).

Application of statistics has broadened far beyond its origin to various areas of research and one among them is the design of experiments (Jacquez 1998). "Design of experiments gathers the maximum amount of information in the lowest number of analyses. In order to optimize a process and maintain repeatability, screening designs are performed" (Vanaja & Shobha Rani 2007). Vanaja and Shobha Rani concluded that screening designs used in early stages of research and development helps the researcher to identify the significant factors for a large-scale simulation with a relatively

small number of runs. As Plackett-Burman design is a multiple of four, it generates the most information for the least amount of work involving the fewest runs. This type of design identifies the factors to be examined more intensely allowing intelligent decision making for future research and development. Applying DoE throughout a pharmaceutical process is the key to quality by design- it maximizes the information content of a small number of experiments. The Plackett-Burman design was developed by R.L. Plackett and J.P. Burman in 1946 and was designed to improve the quality control process that could be used to study the effects of design parameters on the system state so that intelligent decisions can be made.

“Design of experiments (DoE) studies can help develop the process knowledge by revealing relationships, including multivariate interaction, between the variable inputs (e.g., component characteristics or process parameters) and the resulting outputs (e.g., in-process material, intermediates, or the final product). Risk analysis tools can be used to screen potential variables for DoE studies to minimize the total number of experiments conducted while maximizing knowledge gained. The results of DoE studies can provide justification for establishing ranges of incoming component quality, equipment, parameters, and in-process material quality attributes” (FDA 2011: 8). “It is essential that activities and studies resulting in process understanding be documented. Documentation should reflect the basis for decisions made about the process. For example, manufacturers should document the variables studied for a unit operation and the rationale for those variables identified as significant” (FDA 2011: 9).

Design of Experiments is not a new concept. Over the years Plackett-Burman design has been applied to experiments performed in the pharmaceutical industry. Table 2.1 has examples of experiments where the Plackett-Burman design was successfully used as an experimental design screening method.

Table 2.1: Studies performed on pharmaceuticals using Plackett-Burman design of experiments

Article/dissertation title	Journal/Resource	Content
Effective parameters in determining cross-linked dextran microsphere characteristics: screening by Plackett-Burman design of experiments.	Kenari, HS., Alinejad, Z., Imani, M., Nodehi, A. 2013. <i>Journal of Microencapsulation</i> . Posted online 27 March 2013.	Screen the effective parameters in preparing cross-linked dextran microspheres to make them controllable for obtaining microspheres with tunable properties. A Plackett-Burman design of experiments was employed as screening methodology to investigate the effects of the kinetics and process parameters, i.e. the mixing speed and emulsification time on the resulting microsphere characteristics. Increasing dextran concentration in the aqueous phase leads to a significant increase in the mean particle size and decrement on water uptake capacity of the resulting microspheres, respectively.
"Vitamin E" fortified parenteral lipid emulsions: Plackett-Burman screening of primary process and composition parameters.	Alayoubi, A., Nazzal, M., Sylvester, PW., Nazzal, A. 2013. <i>Drug Development and Industrial Pharmacy</i> , 39 (2): 363-373.	The objective of this study was to screen the effect of eight formulations and process parameters on the physical attributes and stability of "Vitamin E"-rich parenteral lipid emulsions. Screening was performed using a 12-run, 8-factor, 2-level Plackett-Burman design . The identification of parameters by a well-constructed design demonstrated the utility of screening studies in the "Quality by Design" approach to pharmaceutical product development.
Preparation and Optimization of Mouth/Orally Dissolving Tablets Using Combination of Glycine, Carboxymethyl Cellulose and Sodium Alginate: A Comparison with Superdisintegrants.	Vora, N and Rana, V. 2008. <i>Pharmaceutical Development and Technology</i> , 13 (3): 233-243.	The purpose of the study was to prepare metoclopramide HCl mouth/orally dissolving tablets (MDTs) using glycine, carboxy methyl cellulose and sodium alginate with sufficient mechanical integrity and disintegration time comparable to superdisintegrants. Application of Plackett-Burman design revealed that concentration of glycine, concentration of carboxy methyl cellulose and tablet crushing strength were found to actively influence the dependent variables (disintegration time in oral cavity, wetting time, porosity and water absorption ratio).

Article/dissertation title	Journal/Resource	Content
Controlled Release Multiparticulate Beads Coated with Starch Acetate: Material Characterization, and Identification of Critical Formulation and Process Variables.	Nutan, MTH., Vaithiyalingam, SR., Khan, MA. 2007. <i>Pharmaceutical Development and Technology</i> , 12 (3): 307-320.	The objectives of the investigation were to prepare and characterize starch acetate with high degree of substitution and to study its prospect as film-forming agent in a controlled-release multiparticulate drug delivery system. As part of the development process by quality by design, the objectives also included identification of critical formulation and process variables that affect the release of a drug. A seven-factor, twelve run Plackett-Burman screening design was used. The main effects on drug release after 12 hours decreased in the following order: coating weight gain, plasticizer concentration, post drying temperature, SA concentration, inlet temperature, post drying time, and atomizing pressure.
Robustness Testing of a Tablet Formulation Using Multivariate Design.	Gabrielsson, J., Sjöström, M., Lindberg, NO., Pihl, AC., Lundstedt, T. 2006. <i>Drug Development and Industrial Pharmacy</i> , 32 (3): 297-307.	45 experiments were carried out to evaluate the robustness of two similar tablet formulations-a product of two strengths-with respect to normal batch-to-batch variation of the excipients and the active pharmaceutical ingredient. Because of the differing amounts of active pharmaceutical ingredients, the two formulations also differ in the amounts of two of the diluents and one of the binders. A Plackett-Burman design was applied to the principle properties. Both formulations were found to be robust under controlled conditions.
Drug-Excipient Compatibility Testing Using a High-Throughput Approach and Statistical Design.	Wytenbach, N., Birringer, C., Alsenz, J., Kuentz, M. 2005. <i>Pharmaceutical Development and Technology</i> , 10 (4): 499-505.	The aim of the research was to develop a miniaturized high throughput drug-excipient compatibility test. Experiments were planned and evaluated using statistical experimental design. To address all factors in this research a considerable number of tests are required. To reduce the number of experiments, fractional designs have been used. Plackett-Burman design was used to study the effect of various excipients, as well as humidity and temperature on pyridoxal hydrochloride degradation. In conclusion, the developed technique enables fast drug-excipient compatibility testing and identification of interactions.

Article/dissertation title	Journal/Resource	Content
A Dual Controlled Gastrointestinal Therapeutic System of Salmon Calcitonin II. Screening of Process and Formulation Variables.	Shah, RB., Nutan, M., Reddy, IK., Khan, MA. 2004. <i>Clinical Research and Regulatory Affairs</i> , 21(3-4): 231-238.	An important aspect of the "Desired State" of the manufacturability as defined by the International Committee of Harmonization is the mechanistic understanding and predictability of dosage forms at the laboratory scale. The accomplishment of that aspect is often preceded by a formulation knowledge and previous history of the project or by screening of the variables to identify the critical ones. A seven-factor-12-run Plackett-Burman screening technique was employed to evaluate the effects of orifice size, coating level, amounts of sodium chloride, Polyox [®] N10 and N80 and Carbopol [®] 934P and 974P on drug release. Factors showing maximum influence on drug release were amounts of Carbopol [®] 934P and Polyox [®] N10 in the drug layer, orifice size and coating level showing negative effects.
Using Experimental Design to Optimize the Process Parameters in Fluidized Bed Granulation.	Rambali, B., Baert, L., Thoné, D., Massart, DL. 2001. <i>Drug Development and Industrial Pharmacy</i> , 27(1): 47-55.	In this study many parameters were screened for a small-scale granulation process for their effect on the yield of granules between 75 and 500 µm and the geometrical granule mean size. First a Plackett-Burman design was applied to screen the inlet air temperature, the inlet flow rate, the spray rate, the nozzle air pressure, the nozzle spray diameter, and the nozzle position. The Plackett-Burman design showed that the key process parameters were the inlet flow rate and the spray rate and probably also the inlet temperature.
The role of Intra- and Extragranular Microcrystalline Cellulose in Tablet Dissolution.	Li, JZ., Rekhi, GS., Augsburger, LL., Shangraw, RF. 1996. <i>Pharmaceutical Development and Technology</i> , 1 (4): 343-355.	The objective of this study was to examine the influence of intra- and extragranular microcrystalline cellulose (MCC) on drug dissolution from tablets made by high-shear granulation. A Plackett-Burman screening design and 2 ³ factorial design were employed to study how drug type, MCC (intra- or extra-), filler type (lactose or dicalcium phosphate), disintegrant type (sodium starch glycolate or croscarmellose sodium) and level, proportion of magnesium stearate, and impeller speed affect tablet hardness, disintegration time, and dissolution. It is concluded that the appropriate distribution of MCC between and within granules may optimize both dissolution and compactibility without changing overall tablet composition.

Article/dissertation title	Journal/Resource	Content
Development of Matrix Controlled Release by Extrusion-Spheronization Technology Using Statistical Screening Design.	Goskonda, SR., Hileman, GA., Upadrashta, SM. 1994. <i>Drug Development and Industrial Pharmacy</i> , 20 (3): 279-292.	The objective of the study was to develop beads with inherent modified release characteristics requiring no subsequent controlled release coating. A Plackett-Burman screening design was employed to isolate critical variables influencing the bead characteristics and drug release. Beads were successfully manufactured according to the screening design and exhibited different dissolution characteristics. Polymer type (Eudragit RS 30 D over Aquacoat ECD-30), polymer concentration and acid concentration significantly retarded drug release. However, increasing acid concentration increased bead friability. In addition to drug concentration, higher polymer concentration, appropriate acid selection and longer residence times afforded maximum capsule fill weights and increased bead density.
A Factorial Approach to High Dose Product Development by an Extrusion/Spheronization Process.	Hileman, GA., Goskonda, SR., Spalitto, AJ., Upadrashta, SM. 1993. <i>Drug Development and Industrial Pharmacy</i> , 19 (4): 483-491.	Extrusion/spheronization technology has been used for preparing high drug-loaded pellets. A Plackett-Burman screening design was employed to investigate product and process parameters affecting final pellet drug content, density and roundness. Microcrystalline Cellulose type and concentration, water concentration, spheronizer speed and residence time and extruder screen size were found to be statistically significant in imparting desirable attributes to the final product. Wet mixing time, extruder feed rate and extrusion rate did not significantly affect the pellet properties.
Quantitation of the Amount and Uniformity of Aqueous Film Coating Applied to Tablets in a 24" Accela-Cota.	Skultety, PF., Rivera, D., Dunleavy, J., Lin, CT. 1988. <i>Drug Development and Industrial Pharmacy</i> , 14 (5): 617-631.	A simple technique was developed to quantify the amount of coating solution applied to individual tablets. A Plackett-Burman experimental design was used to determine the processing parameters in a 24" Accela-Cota which can influence the homogeneity of film coating applied to tablets. The processing parameters that were found to affect the film coating were: coating drum speed; amount of aqueous film coating liquid applied; and the spray pattern. The starting location of the tablets in the tablet bed did not affect the amount of coating applied or the variability of the coating. Different size and shape tablets were found to behave similarly as to what processing parameters were significant in the amount of coating applied and the variability between tablets.

Article/dissertation title	Journal/Resource	Content
Evaluation of the Loss of Propylene Glycol During Aqueous Film Coating.	Skultety, PF., Sims, SM. 1987. <i>Drug Development and Industrial Pharmacy</i> , 13 (12): 2209-2219.	A Plackett-Burman study was used to determine the coating factors which can affect the loss of propylene glycol, a common water soluble plasticizer used on aqueous film coating, during the film coating process. Analysis of the data shows that the amount of the propylene glycol in the film was 81 to 96 % less than the theoretical value when considering the amount of the propylene glycol in the aqueous film coating liquid. The loss of propylene glycol was independent of the variables studied. The loss of propylene glycol was also shown to occur in the Accela-Cota during the coating of tablets.
Buccoadhesive films for once-a-day administration of rivastigmine: systematic formulation development and pharmacokinetic evaluation.	Kapil, R., Dhawan, S., Beg, S., Singh, B. 2013. <i>Drug Development and Industrial Pharmacy</i> , 39 (3): 466-480.	A Plackett-Burman Design was employed for screening of significant formulation and process variables involved in the development of buccoadhesive films. Implementation of the design helped in identifying the most significant factors for further detailed investigation with minimum experimentation, thus saving considerable time, effort and materials.
Formulation of an anti-tuberculosis drug delivery system.	Du Toit, LC. 2007. Masters dissertation, vi, 266.	Statistical experimental design (Plackett-Burman), implementing response-surface methodology was pivotally instituted on the multiparticulate forms for the identification of critical formulation and processing variables for the development of the optimum enterosoluble and reconstitutable multiparticulate systems for delivery to the patient as the preferred multiparticulate two-drug FDC.

2.3 Sampling techniques

In establishing guidance for good mixing parameters, one needs to keep in mind that it is also dependent on reliable, accurate, analytical data for which sampling is an integral and pivotal issue (Venables & Wells 2001).

Once samples have been collected from the bowl of the granulator, by sampling methods, the mean value and sample variance are determined and then often used in a mixing index. Many mixing indices are available; however, there is no general mixing index, so choice of index is left to the

individual investigator (Poux et al., 1991 cited in Alexander & Muzzio 2005). Once a measure of mixedness has been defined, it is then tracked over time until suitable homogeneity is achieved. Ideally, this minimum level of variance would stay relatively constant over a sufficiently long time. This procedure is simple in concept, but many problems have been associated with characterisation of granular mixtures (Muzzio et al., 1997 cited in Alexander & Muzzio 2005). One dangerous assumption is that a small number of samples can sufficiently characterize variability throughout the blend. Furthermore, sample size can have a large impact on apparent variability. Samples that are too small can show exaggerated variation, while too large a sample can blur concentration gradients. A sufficient number of samples should be taken representing a large cross-section of the blender volume. The sample size for pharmaceutical products is ideally of the scale of the unit dose and in the case of small unit doses the goal should be to sample at a size within the resolution of the sample thief (Hickey & Ganderton 2001).

Sampling thief probes remain the most commonly employed instrument for sampling powders for data gathering. (Muzzio et al., 1999). These instruments have however been demonstrated to sometimes induce large sampling errors coming from poor flow into the thief cavity or sample contamination (carryover from other zones of the mixer) during thief insertion (Muzzio et al., 1997 cited in Alexander & Muzzio 2005).

An end-sampling thief was used successfully to collect all samples in the study conducted on blenders by Sudah et al. (2002). The sampler has hollow tubes with one end filed to a sharp edge, which minimise bed disturbance when thrust into the mixture.

Acceptance criteria for blend assay used during Bozzone's (2001) research study: 10 samples from powder blends, and individual assay % RSD < 5.0 %. Demonstration of mix and content uniformity for exhibit and/or validation batches: from the blend, sample at least 10 locations, with at least 3 replicates from each location. Assay 1 per location. Blend sample criteria: RSD \leq 5.0 % and all individuals are within 10 % (absolute) of the mean of the results. Absolute as used to define the acceptable range (\pm 10 %) in which individual blend sample values must fall is dependent of the value of the mean. For example, if the mean of all blend samples is 95.0 %, the absolute range is 85 – 105 %. This was for a study performed on blenders. Stratified sampling of blend and dosage units to demonstrate adequacy of mix for powder blends has also been suggested (Prescott & Garcia 2001).

3. METHODOLOGY

By using a Fishbone diagram for risk analysis in figure 2.1, the factors that are more likely to have an impact on the outcome were assessed. Operating parameters were be the main focus of this project and the influence it has on the homogeneity of a mix. Two of the factors that were assessed include mixer/impeller blade and chopper blade speeds. The third factor that was assessed in this study includes mixing time. Different ratios of two powders were used in these experiments.

With this project it was established which aspect has the most important impact on the desired quality attribute, which is homogeneity of the mixture, through varying four of the parameters (mixing time, mixer/impeller blade speed, chopper blade speed, different ratios of two powders). These factors were investigated in a design of experiments.

3.1 Materials

Two raw materials of different density and particle sizes were mixed together: lactose (DMV Fonterra Excipients, Germany) and enalapril (Zhejiang, China). Enalapril was chosen as one of the raw materials as it can be quantified via assay testing and can therefore indicate the adequacy of the mixture. The type of powders were the same for the experiments, however the impact on three different ratios of the two powders were assessed.

3.1.1 Logistics

The experiments were performed in a *Research Laboratory* at a pharmaceutical company. The laboratory testing was performed in the *analytical laboratory* at the pharmaceutical manufacturing company. This is a MCC (Medicines Control Council) approved facility. MCC is the regulatory authority in South Africa. Mixing and testing were performed by the researcher.

The temperature was maintained at 22.5 °C (\pm 2.5 °C).

The relative humidity was maintained at less than 45 %.

3.1.2 Equipment

A *Saral Rapid mixer and wet Granulator* (Saral Engineering Company, India) were used. The model is: RMG 10 Ltr. (R&D Model). The main impeller blade runs in the horizontal plane and the chopper blade runs in the vertical plane. It is a precise machine performing dry mixing and wet granulation in the same bowl. The *main impeller* blade range is 1–205 rpm and the *chopper* blade range is 0-2880 rpm (Saral, 2006). The two blades were operated at a low, medium and high speed. The function of the mixer blade is to mix and the function of the chopper blade is to break up lumps. Dry *mixing time* suggested by the manufacturer for this machine is 2 – 4 minutes. Working capacity is 4 kg batch size (assuming a bulk density of 0.5). Overflow capacity is 10 kg. The equipment has been qualified. This equipment is bottom-driven. Figure 3.1 is an illustration of the equipment and figure 3.2 is an illustration of the blades.



Figure 3.1: *Saral Rapid Mixer and wet granulator R&D model*



Figure 3.2: *Mixer blades of Saral Rapid Mixer and wet granulator R&D model*

3.1.3 Formulations of powder mixtures for analysis

Formulations consisted of lactose, which is a diluent/bulking agent and enalapril maleate. The two materials need to be evenly distributed throughout the mixture to ensure that the end-product has the correct amount of each of the ingredients per tablet as per the label claim. The high shear mixer was loaded with the lactose first and then the enalapril on top of the lactose in the middle of the bowl, before switching on the mixer. The formulations tested were as per table 3.1.

Table 3.1: Three formulations with different ratios of two powders used in experiments

Formulation	0.2 % w/w Enalapril ratio	10.1 % w/w Enalapril ratio	20.0 % w/w Enalapril ratio
Enalapril maleate	0.008 kg	0.404kg	0.800 kg
Lactose monohydrate	3.992 kg	3.596 kg	3.200 kg
Total	4.000 kg	4.000 kg	4.000 kg

Lot numbers for batches of raw material used in experiments:

Lactose: B018216 (used in Batch 1)
 B021515 (used in Batches 2 and 3)

Enalapril: X111972 (used in Batch 1)
 B022065 (used in Batches 2 and 3)

Refer to Certificates of analysis for these batches in Appendix A.

From the certificates it can be seen that the bulk density and particle size for the materials were as listed in table 3.2.

Table 3.2: Particle size and bulk densities for materials used in the experiments

Lot numbers	Particle size	Bulk density
B018216	78-86 % < 31 µm 98 % < 41 µm	0.781 g/ml
B021515	78-86 % < 75 µm 98 % < 16 µm	0.836 g/ml
X111972	90 % < 20 µm	-
B022065	90 % < 20 µm	-

The lactose is the biggest part of the mixture and therefore the bulk density of the lactose is mentioned. The enalapril is a small portion of the mixture and would not have a great impact on the bulk density of the mixture.

3.2 Methods

3.2.1 Statistical Experimental Design

Design of Experiments was used. The project followed a 2^4 Plackett-Burman factorial experimental design. The designs were developed using the Essential Experimental Design[®] program EREGRESS Version 2.209 (2006-Copyright). The program is freeware and was developed by R.P. Yeater, D.D. Steppan and J. Werner.

In statistics, regression analysis is a collective name for techniques for the modelling and analysis of numerical data consisting of values of a dependent variable (also called response variable or measurement) and of one or more independent variables (also known as explanatory variables or predictors).

The dependent variable in the regression equation is modelled as a function of the independent variables, corresponding parameters ("constants") and an error term.

Linear regression was used to determine the extent to which there is a linear relationship between a dependent variable and one or more independent variables.

The following statistics were used:

- Summary
- p value
- R^2 = % predicted by model correlation
- Analysis of variance
- Critical significance?
- Response surfaces plots
- 95 % CI- 5 % chance of error
- Correlation matrix

- Current value- it calculate what the best parameters will be to get the lowest % RSD. The optimal parameters can be predicted.
- Predicted vs actual.

In experimental designs, a number of cause factors and response variables (dependent variable) are selected. The relationship between cause variables and response is established mathematically. The optimal response variable can then be obtained by selecting the optimal cause variables.

The factors tested and thus the four input parameters are listed in table 3.3. The output parameter is % Relative Standard Deviation (% RSD) for assay tested from 7 samples taken from predetermined points in the bowl after the mixing time for the specific experiment was completed. A lower % RSD would indicate a more homogenous mix. A high % RSD would indicate that the two powders are not yet well distributed throughout the mixture.

Table 3.3: High, medium and low level settings for high speed mixer/granulator in the Plackett-Burman Screening Design

Factor	Factor Level			Unit	Outcome
	Low (-1)	Medium (0)	High (+1)		
Enalapril concentration	0.20	10.10	20.00	% w/w	Assayed seven samples from each experiment. Determined % RSD (Relative Standard Deviation) for each set of seven samples
Speed of blades					
Main Impeller	50	125	200	rpm	
Chopper	1000	1900	2800	rpm	
Mixing time	0.5	2.0	3.5	minutes	

Table 3.4 lists the experimental design, detailing the levels of the formulations to be tested.

Each experiment was performed on three batches.

Table 3.4: 2^4 two-level Plackett-Burman Design table showing experimental runs

Plackett-Burman Design, Resolution 3 4 Factors 12 model runs and 2 Center points (experiments 4 & 6 and 5 & 12) Linear Model with 5 terms <u>Equation:</u> Response = $b_0 + b_1 \cdot \text{enalapril, \% w/w} + b_2 \cdot \text{Impeller, rpm} + b_3 \cdot \text{Chopper, rpm} + b_4 \cdot \text{Time, minutes}$

Exp #	Enalapril ratio, %w/w	Impeller speed, rpm	Chopper speed, rpm	Time, minutes
1	0.20	50	1000	0.5
2	0.20	200	2800	3.5
3	0.20	200	1000	0.5
4	10.10	125	1900	2.0
5	20.00	200	1000	3.5
6	10.10	125	1900	2.0
7	0.20	200	2800	0.5
8	20.00	50	2800	3.5
9	0.20	50	2800	3.5
10	0.20	50	1000	3.5
11	20.00	200	2800	0.5
12	20.00	200	1000	3.5
13	20.00	50	1000	0.5
14	20.00	50	2800	0.5

As yet, no reliable techniques for on-line measuring of composition have been developed; hence, powder mixtures are usually quantified by removing samples from the mixture. To determine blending behaviour over time, the blender is stopped at fixed *intervals for sampling*; the process of interrupting

the blend cycle and repeated sampling may change the state of the blend (Alexander & Muzzio 2006). For this study, the mixing was not interrupted for sampling. The powders were mixed for the full time and was then sampled.

3.2.2 Sampling of powder mixtures that was analysed

For each experiment *seven samples* were taken from seven different points in the bowl. It was taken at the top and bottom of the bowl at three points in the bowl and one sample was taken from a point in the top middle of the bowl. Figure 3.3 illustrates the sampling points in the bowl. Samples were taken in duplicate, but only one sample per location was tested. The seven samples were sufficient to represent a large cross-section of the entire bowl surface.

Sample size was ± 400 mg.

For blend uniformity, sample size must be at most 3 unit dosages. This criteria originated from the U.S. vs. Barr Laboratories court case (United States of America, Plaintiff, vs. Barr Laboratories, Inc. et al., Defendants. 1993) that took place in New Jersey in 1993. "The proper blend sample size for content uniformity testing is three times the run weight of the finished product" (Buckley & Associates 2004).

The sample size is approximately 3 times the weight of a tablet ($150 \text{ mg} \times 3 = 450 \text{ mg}$).

Enalapril tablets are small tablets. Bulk density of powders ± 0.79 g/ml.

$$450 \text{ mg} / 0.79 \text{ g/ml} = 0.57 \text{ ml}$$

$$0.5 \text{ cc} = 0.5 \text{ ml used}$$

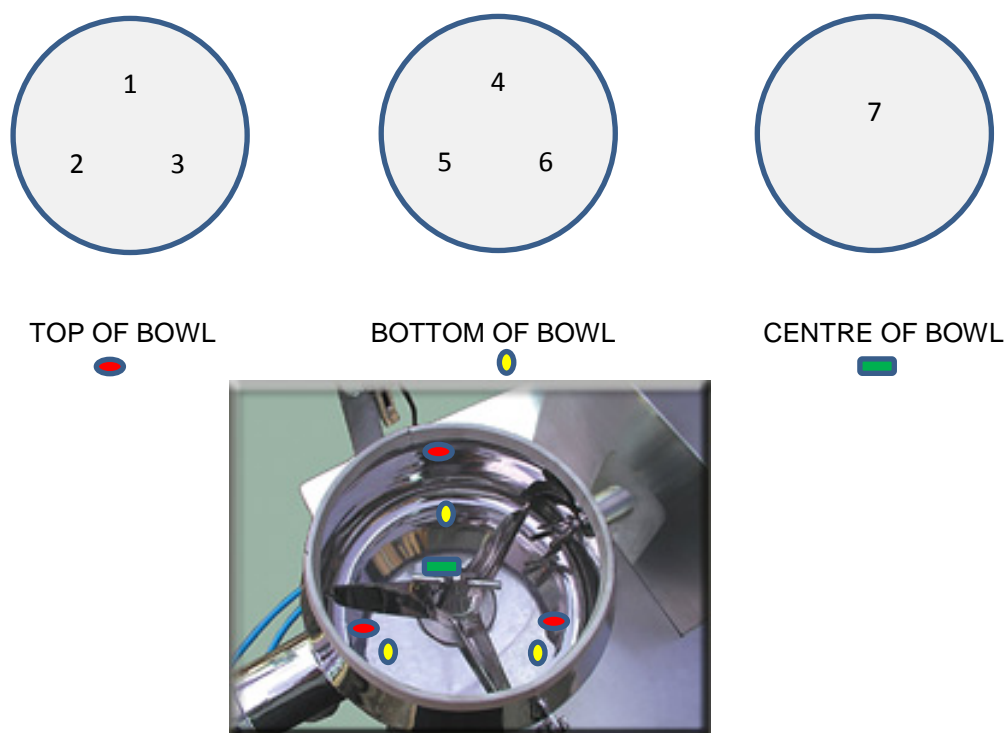


Figure 3.3: Sampling points in bowl of Saral Rapid Mixer and wet granulator R&D model

3.2.2.1 Sampling equipment

A sampling thief with one disc was used for sampling of powders. All samples were taken by the researcher. This was to ensure sampling consistency. The correct volume insert was chosen.

The volume insert was inserted into the lowest point of the inner rod of the sampling thief. The inner rod was slid into the outer sleeve. The angle of insertion of the sampling thief into the product was 45 degrees and it was inserted slowly into the powder mixture. The handle was turned to expose the sampling cell to the product. The handle was turned so that no sampling inserts were exposed. The sampling thief was then removed from the product. The sample was placed into a bottle and placed onto the table from where the HPLC testing was performed. This was to avoid any further mixing that could have taken place.

Literature suggests that this tool can be useful for free-flowing powders. Even though there are disadvantages to the sampling thief, it was chosen as the sampling equipment for these experiments as the same thief was used to sample all samples. It would be sufficient for the purpose of this project. The powders have proven to have enough flowability. Due to the fact that the variability for

the % RSD among the three batches for each experiment were not extensive, also proves that the sampling was adequate for the study. Figure 3.4, 3.5 and 3.6 illustrate the sampling tool used.



Figure 3.4: Sampling thief



Figure 3.5: Sample insert into sampling thief

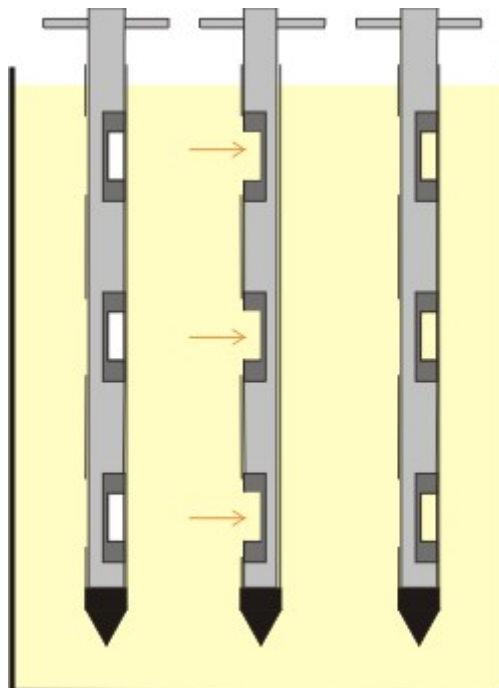


Figure 3.6: Sketch showing how the sample thief works

3.2.3 Calculation of %RSD

The 7 samples taken from each experiment from the three batches/ lots were assayed. The mean value and percentage Relative Standard Deviation (% RSD) was then calculated for each experiment. The USFDA issued a guidance for industry on 'Powder blends and finished dosage units – stratified in-process dosage unit sampling and assessment' (US Food and Drug Administration 2003). This guidance is intended to assist manufacturers of human drug products in meeting the requirements of 21 CFR 211.110 for demonstrating the adequacy of mixing to ensure uniformity of in-process powder blends and finished dosage units. This guidance describes the procedures for assessing powder mix adequacy, correlating in-process dosage unit test results with powder mix test results, and establishing the initial criteria for control procedures used in routine manufacturing. The recommended criteria to use for a powder blend (in a blender) is to assay one sample per location (number of samples ($n \geq 10$)). The Relative Standard Deviation of all the individual results should be $\leq 5.0\%$. All the individual results should be within 10.0% (absolute) of the mean results. Note that this is the criteria for a powder blend that could also be applied to the dry mix.

3.2.4 Assay method for enalapril

The USP method was used to assay the samples via *High-Performance Liquid Chromatography*. Chemical experiments in a laboratory represent one of the purest forms of experimental type research (Walliman 2005).

Due to the fact that a USP method is used for the analysis, there was no need to perform a full validation on the method. USP methods are robust methods. Pharmacopoeial methods require partial validation (USFDA 2014). Partial validation included: system suitability and specificity. The method used is a stability indicating method. The method is hence capable of separating the API from impurities and degradation products. Specificity, System suitability, Linearity, Accuracy and Method precision were performed on this method. System suitability (precision) was tested during the experiments and passed during all testing that were performed for this project.

3.2.4.1 Apparatus and operating conditions

The following instrument was used for testing of the samples:

High-Performance Liquid Chromatograph (Waters Alliance HPLC System, 2695 Separations Module with a Waters 2487 Dual wavelength Absorbance Detector and Waters Alliance HPLC System, 2695 Separation Module with a Waters 2489 dual wavelength absorbance detector, United States).

The liquid chromatograph was equipped with a high pressure pump and a variable wavelength UV/VIS detector with the wavelength set at 215 nm. The auto-sampler on the equipment was set to inject 50 µl. The equipment has an integrator system. The column used was a Platinum EPS, C8 (5 µm) (25 cm x 4,6 mm i.d.) column with a temperature at 50 °C.

The following instrument was used for weighing of the samples:

Balance (Mettler Toledo Top Loader balance and LC-P43 printer, Columbus)

The following equipment was used for mixing and for weighing of powders:

Measuring cylinders, volumetric flasks and beakers (Glassco Laboratory Equipments, India)

The solvent consisted of a buffer solution, which is explained below.

The mobile phase was as follows: A mixture of acetonitrile (Merck, USA) : pH 2.2 buffer solution (25 : 75), which was filtered and degassed.

The buffer solution was prepared as follows: 1.38 g of monobasic sodium phosphate (Merck, USA) (Mw = 137.99 g/mol) was dissolved in 800 ml of water. The solution was adjusted with phosphoric acid (Merck, USA) to a pH of 2.2 and diluted with water to 1000 ml. It was then mixed.

The mobile phase was prepared as follows: 250 ml of acetonitrile was mixed with 750 ml buffer. This mix was filtered and degassed.

The flow rate used was 2.0 ml/min. Enough of the mobile phase was prepared for all samples tested.

The amount was calculated for each sample using \pm 50 ml of mobile phase.

3.2.4.2 Preparation of standard solution

The standard solution was prepared as follows:

20 mg of enalapril maleate reference standard was weighed and put into a 100 ml volumetric flask.

Solvent (buffer) was added to the flask and it was put in a sonicator and set to sonicate for 15

minutes. This solution was then allowed to cool down to room temperature. The solution in the flask was diluted to volume with solvent (buffer solution).

NOTE: Due to reference standards being expensive, an in-house working standard was used. This working/secondary standard was qualified against the USP pharmacopoeia/primary standard. The standard used was within its expiry period and had dried basis assay of 100.0 % and as is assay of 99.6 %.

3.2.4.3 Preparation of recovery standard solution

20 mg of enalapril maleate reference standard was weighed into a 100 ml volumetric flask. Solvent (buffer) was added to the flask and it was put in a sonicator and set to sonicate for 15 minutes. This solution was then allowed to cool down to room temperature (21 degrees Celcius). The solution in the flask was diluted to volume with solvent.

NOTE: The scales/balances used were calibrated and maintained according to standard operating procedures.

3.2.4.4 Preparation of sample solution (for all 14 experiments' samples)

The sample solution (done for all 14 experiments' samples-from 3 batches) was prepared as follows: 150 mg of sample was added to each of the seven 100 ml volumetric flasks. 25 ml of solvent (buffer) was added to each flask and the flask was shaken manually. The solvent was then diluted to just below the neck of the flask. It was put in a sonicator and set to sonicate for 15 minutes. The solution was then allowed to cool down to room temperature. The solution in the flask was diluted to the mark with solvent and mixed well.

3.2.4.5 Assay Procedure for enalapril

Once all samples and the mobile phase were prepared, the equipment was set up.

A run time of 10 minutes for the standard and the samples were employed.

The HPLC column was equilibrated with the mobile phase for 30 minutes. The standard solution was injected five times and the average of the peak area results were calculated.

A system suitability test was performed on the five standard injections and the parameters were

calculated and complied to the USP: the relative standard deviation of the peak areas due to the enalapril peak for 5 replicate injections was not more than 2 %.

The recovery standard solution was injected twice and the recovery was calculated using the calculation for % Recovery Control (equation 1). The % recovery was between 98.0 – 102.0 % with respect to the enalapril peak.

50 µl of the assay sample solution was injected twice and the average of the peak area results was calculated. The assay results were then calculated as specified in equation 2.

The standard solution was injected twice at the end of the run. The relative deviation of the peak areas due to the enalapril peak for all standards was not more than 3.0 %.

3.2.4.6 Calculations

The calculations used were as follows:

3.2.4.6.1. % Recovery Control

$$\% \text{ Recovery Control} = \frac{P_{\text{rec}} \times \text{mass}_{\text{std}} \times 100}{P_{\text{std}} \times \text{mass}_{\text{rec}}} \quad \text{Equation 1}$$

where:

P_{rec} = area of the relevant peak in the recovery standard solution

P_{std} = area of the relevant peak in the standard solution

mass std = mass of relevant standard taken to prepare the standard solution, expressed in mg

mass rec = mass of relevant standard taken to prepare the recovery standard solution, expressed in mg

3.2.4.6.2 % m/m enalapril maleate

% m/m enalapril maleate

$$= \frac{\text{the area of the enalapril peak in the sample solution} \times \text{mass}_{\text{std}} \times 50 \times C \times 100}{\text{the area of the enalapril peak in the standard solution} \times 100 \times \text{mass}_{\text{sam}} \times 100} \quad \text{Equation 2}$$

where:

mass std = mass of enalapril maleate reference standard taken to prepare the standard solution, expressed in mg.

C = potency of enalapril maleate reference standard, expressed as a percentage (100 %).

3.2.4.7 Typical chromatograms

Figure 3.7 illustrates a typical chromatogram for enalapril. The chromatogram was developed using the above chromatographic conditions.

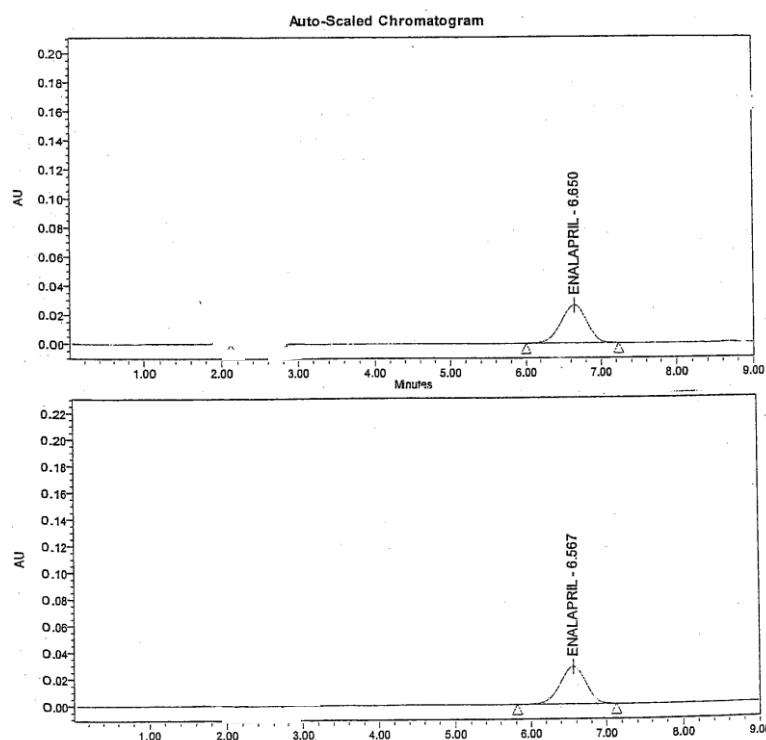


Figure 3.7: Chromatograms for enalapril

The *outcome* was measured as % RSD of the 7 samples from the bowl per experiment, indicating the homogeneity of the blend. A lower % RSD is indicative of a more homogenous blend. The blend of the specific experiment is homogenous if the active content of all 7 assayed samples are within 10 % of the mean results and the relative standard deviation (RSD) of the 7 results is less than or equal to 5 %. The target % m/m was either 0.20 %, 10.10 % or 20.00 %, depending on the experiment.

% RSD was calculated as per equation 3:

$$\% \text{ Relative Standard Deviation} = [(\text{std deviation}) / (\text{mean})] \times 100 \% \quad \text{Equation 3}$$

The *importance of each factor on homogeneity of the mix* was established as well as the parameters (mixing speeds and time) that gave rise to a more homogenous mix.

Results obtained could be used by the pharmaceutical company to *compare to current parameters* used in the pharmaceutical company. This data will be obtained from reports containing mixing parameters used for products mixed in this equipment. The outcome might be that the optimal mixing parameters are different from the current parameters in use, which can prompt the pharmaceutical company to change the parameters or to use different operating parameters for future products manufactured using this equipment. It might also be found that the parameters, which are currently used, are the optimal parameters, and then this study will serve as confirmation and a reference that can be used during future audits. It can also provide answers to possible audit questions and serve as proof to auditors that the parameters used have been well researched.

The occupancy in a mixer/granulator bowl can be determined by using the bulk density for a powder sample. For this report we are only looking at enalapril maleate and lactose of constant bulk densities.

3.3 Statistical analysis/data analysis

A “screening design” refers to an experimental design that is applicable when a large number of potential causative factors need to be examined to find the most important few that may have an effect on one or more responses of interest. Screening designs may be derived from highly fractionated factorial designs. Plackett and Burman devised orthogonal arrays useful for screening that yield unbiased estimates of all main effects in the smallest design possible (Murphy 2010).

Response surface methodology (RSM) was used to analyse the data. RSM is a collection of mathematical and statistical methods that are used to develop, improve or to optimize a product or process. It comprises of statistical experimental designs, regression modelling techniques, as well as optimization methods. The application of RSM involves experimental situations in which several independent variables potentially influence one (or more) response variable. The independent variables are controlled by the experimenter, in a designed experiment, whereas the response variable is an observed output of the experiment (Myers 2010). The designs have been developed by

the graphic software: The Essential Experimental Design[®] program EREGRESS Version 2.209 (2006-Copyright). The statistical analyses of the measured response parameters have also been carried out with this software.

3.4 Limitations of the design

There are some downsides to the use of Plackett-Burman design and therefore this design can be used as a starting point. This design may be followed with more detailed experimentation. Plackett-Burman design is very helpful when one has a lot of potential factors and it needs to be established which are the vital few that will assist in solving problems and can be used to make decisions. This design is a useful tool to use when one wants to use design of experiments to learn as much as possible from the smallest amount of data. It is a tool that can be used to assist in ruling out the unimportant factors. It is often called a screening design. Once the significant factors have been established, the interaction between factors could be established by doing more experiments (eg. full factorial design).

4. RESULTS

14 experiments were performed on three lots/batches of lactose and enalapril mixtures. Seven samples were taken from the bowl in duplicate during the different experiments and assay testing was performed on these samples. The percentage standard deviation was calculated for the assay results of the seven samples. The combination of results of seven samples with the lowest percentage relative standard deviation indicates the best mixing parameters for the dry mixing.

Refer to Appendix B for all HPLC results obtained for all experiments and Appendix C for all results from all experiments. Table 4.2 outlines a summary of the results for the experiments.

The % standard deviation for the output of the three batches varied from 0.43 – 23.53 %.

The experiments with the lowest % RSD include: experiment 2 (1.71 %), experiment 8 (1.83 %), experiment 12 (2.45 %), experiment 4 (3.10 %), experiment 5 (3.19 %), experiment 11 (3.86 %), experiment 6 (4.30 %) and experiment 9 (10.70 %).

The % RSD values for experiments 1 (62.33 %), 10 (52.11 %), 13 (59.06 %) and 14 (50.78 %) are the highest.

The 2 centre points/ markers were experiments with numbers:

- 4 and 6 (all factors are at their central values) and
- 5 and 12.

The results for these experiments are outlined in table 4.1.

The % RSD values obtained for these markers are similar. This indicates repeatability/reproducibility of the project. Reproducibility is one component of the precision of a measurement or test method.

Table 4.1: *Experiment centre points*

Exp #	Enalapril ratio, w/w	Impeller, rpm	Chopper, rpm	Time, minutes	% RSD
4	10.10	125	1900	2	3.10
6	10.10	125	1900	2	4.30
5	20.00	200	1000	3.5	3.19
12	20.00	200	1000	3.5	2.45

Table 4.2: Output for three batches

Exp #	Enalapril conc (% ^m / _m)	Impeller Speed (rpm)	Chopper Speed (rpm)	Time (minutes)	% RSD for 7 samples				
					Batch 1	Batch 2	Batch 3	Average of three batches	Std dev for %RSD of the three batches
1	0.20	50	1000	0.5	54.09 %	88.88 %	44.03 %	62.33%	23.53%
2	0.20	200	2800	3.5	2.75%	0.64%	1.75%	1.71%	1.06%
3	0.20	200	1000	0.5	21.76%	51.49%	34.88%	36.04%	14.90%
4	10.10	125	1900	2.0	2.39%	4.60%	2.31%	3.10%	1.30%
5	20.00	200	1000	3.5	2.28%	5.29%	1.99%	3.19%	1.83%
6	10.10	125	1900	2.0	4.69%	4.36%	3.84%	4.30%	0.43%
7	0.20	200	2800	0.5	25.82%	24.80%	20.68%	23.77%	2.72%
8	20.00	50	2800	3.5	1.43%	1.65%	2.41%	1.83%	0.51%
9	0.20	50	2800	3.5	8.40%	7.99%	15.72%	10.70%	4.35%
10	0.20	50	1000	3.5	44.78%	74.41%	37.14%	52.11%	19.69%
11	20.00	200	2800	0.5	2.54%	3.35%	5.69%	3.86%	1.64%
12	20.00	200	1000	3.5	2.10%	3.33%	1.92%	2.45%	0.77%
13	20.00	50	1000	0.5	46.05%	63.10 %	68.03%	59.06%	11.53%
14	20.00	50	2800	0.5	55.37%	48.60 %	48.37%	50.78%	3.98%

4.1 Statistical analysis

The regression output for the model equation (equation 4)

$$\% \text{ RSD} = b_0 + b_1 * \text{Concentration, \%w/w} + b_2 * \text{Impeller, rpm} + b_3 * \text{Chopper, rpm} + b_4 * \text{Time, minutes}$$

Equation 4

is shown in table 4.3.

From the p values in table 4.3, it is clear that there are three significant input variables (with p value < 0.05). The order of significance from highest to lowest is: impeller blade speed, mixing time, chopper blade speed. Concentration is not statistically significant. This confirms the findings made by the following researchers:

- Kaufman in 1962: active concentration did not affect the homogeneity of a mix.
- Venables & Wells in 2001: drug:excipient ratio has no effect.
- Sudah et al. in 2002: the concentration of the active component did not affect the outcome of the mixing process.

Backward elimination was performed, to eliminate the (not significant) concentration input variable.

The results for the backward elimination are shown in table 4.4.

The linear regression model equation is depicted in equation 5:

$$\% \text{RSD} = 85.30 - 0.184 * \text{Impeller} - 0.01135 * \text{Chopper} - 9.103 * \text{Time.}$$

Equation 5

Table 4.3: Regression output for model $\% RSD = b_0 + b_1 \cdot \text{Concentration, \%w/w} + b_2 \cdot \text{Impeller, rpm} + b_3 \cdot \text{Chopper, rpm} + b_4 \cdot \text{Time, minutes}$

Summary							
R						0.898	
R ²						0.806	
R ² adjusted						0.720	
Standard Error						12.81	
# Points						14	
PRESS						2989.72	
R ² for Prediction						0.607	
Durbin-Watson d						2.242	
First Order Autocorrelation						-0.217	
Collinearity						1.000	
Coefficient of Variation						56.891	
Precision Index						178.894	
ANOVA							
Source	SS	SS%	MS	F	F Signif	Df	
Regression	6136.3	81	1534.1	9.349	0.00289	4	
Residual	1476.8	19	164.09			9	
LOF Error	1475.9	19 (100)	210.84	424.3032	0.00235	7	
Pure Error	0.994	0 (0)	0.497			2	
Total	7613.2	100				13	
		p value	Std Error	-95%	95%	t Stat	VIF
b0	90.87	3.93454E-05	12.21	63.24	118.50	7.440	
b1, Concentration	-0.551	0.174	0.374	-1.396	0.294	-1.476	1.000
b2, Impeller speed	-0.184	0.00465	0.04931	-0.296	-0.07267	-3.736	1.000
b3, Chopper speed	-0.01135	0.02207	0.00411	0.02064	-0.00205	-2.761	1.000
b4, Mixing time	-9.103	0.00498	2.465	-14.68	-3.526	-3.692	1.000

Table 4.4: Regression output for model $\%RSD = b_0 + b_1 \cdot \text{Impeller, rpm} + b_2 \cdot \text{Chopper, rpm} + b_3 \cdot \text{Time, minutes}$

Summary							
R						0.871	
R ²						0.759	
R ² adjusted						0.687	
Standard Error						13.54	
# Points						14	
PRESS						3266.21	
R ² for Prediction						0.571	
Durbin-Watson d						2.358	
First Order Autocorrelation						-0.213	
Collinearity						1.000	
Coefficient of Variation						60.149	
Precision Index						20.187	
ANOVA							
Source	SS	SS%	MS	F	F Signif	Df	
Regression	5778.9	76	1926.3	10.50	0.00196	3	
Residual	1834.3	24	183.43			10	
LOF Error	1590.4	21 (87)	318.08	6.5211	0.03016	5	
Pure Error	243.88	3 (13)	48.78			5	
Total	7613.2	100				13	
		p value	Std Error	-95%	95%	t Stat	VIF
b0	85.30	3.97015E-05	12.28	57.94	112.67	6.945	
b1	-0.184	0.00541	0.05213	-0.300	-0.06806	-3.534	1.000
b2	-0.01135	0.02597	0.00434	-0.02102	-0.00167	-2.612	1.000
b3	-9.103	0.00580	2.606	-14.91	-3.295	-3.492	1.000

The data in table 4.4 is used for predicting the optimal values of the impeller blade and chopper blade speeds and mixing time to get the lowest %RSD.

As one increases the time of mixing, the % RSD moves to a minimum. As the impeller speed increases, there is a slight decrease in the % RSD. As the chopper blade speed is increased, the % RSD also decreases slightly. The results suggest that if the impeller blade speed is set to its

maximum and the mixture is mixed for longer at a higher chopper blade speed, the more homogenous the resultant mixture will be.

Table 4.5: Optimal input parameters to achieve the minimum % RSD

Term	Impeller,rpm	Chopper, rpm	Time,minutes
Data Min	50	1000	0.5
Data Avg	125	1900	2
Data Max	200	2800	3.5
Cur Value	191	2002	3.01

The % RSD for the Current Values (which were adjusted to give a minimum %RSD) in table 4.5 is 0.007462 %.

4.1.1 Statistical Significance

To determine if the regression model fits (the whole model) is statistically significant; a statistical test must be conducted. In this case the F-test.

A probability value (p-value) is produced which indicates statistical significance if this calculated p-value is smaller than 0.05.

4.1.1.1 The overall model:

The p-value from the F-test is less than 0.05($F_{3,13}=10.50;p=0.00196$) indicating a significant fit of the model at a 95% level of confidence.

The equation that fits this model is shown in equation 6.

$$\%RSD = 85.30 - 0.184 * \text{Impeller} - 0.01135 * \text{Chopper} - 9.103 * \text{Time} \quad \text{Equation 6}$$

4.1.1.2 Individual predictors:

To assess the significance of the individual independent variables, t-tests and the individual p-values were used.

The input factor impeller blade speed has a statistically significant influence on % RSD at a 95% level of confidence with a p-value below 0.05 ($t=-3.34$; $p=0.00541$). The input factor Time also has a significant influence on %RSD at a 95% level of confidence with a p-value below 0.05 ($t=-3.492$; $p=0.00580$) as well as the input factor chopper blade speed below 0.05 ($t=-2.612$; $p=0.02597$). The 2 factors that have the most significant impact on the end result were impeller speed and mixing time, as the p-value for these two parameters are much smaller than that for the chopper blade speed.

4.1.2 Response surface plots

Response surface plots were drawn up in figures 4.1 – 4.3 which show the relationships between the statistical significant parameters.

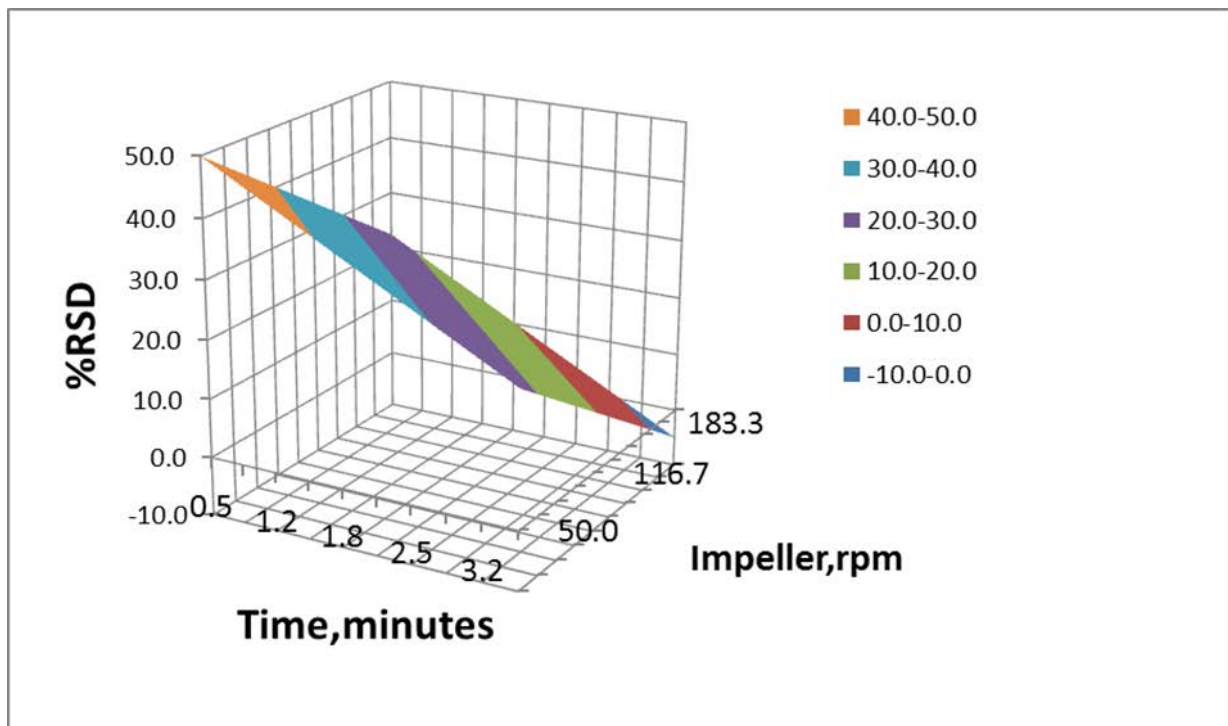


Figure 4.1: Response surface plot of estimated effects of mixing time and impeller blade speed

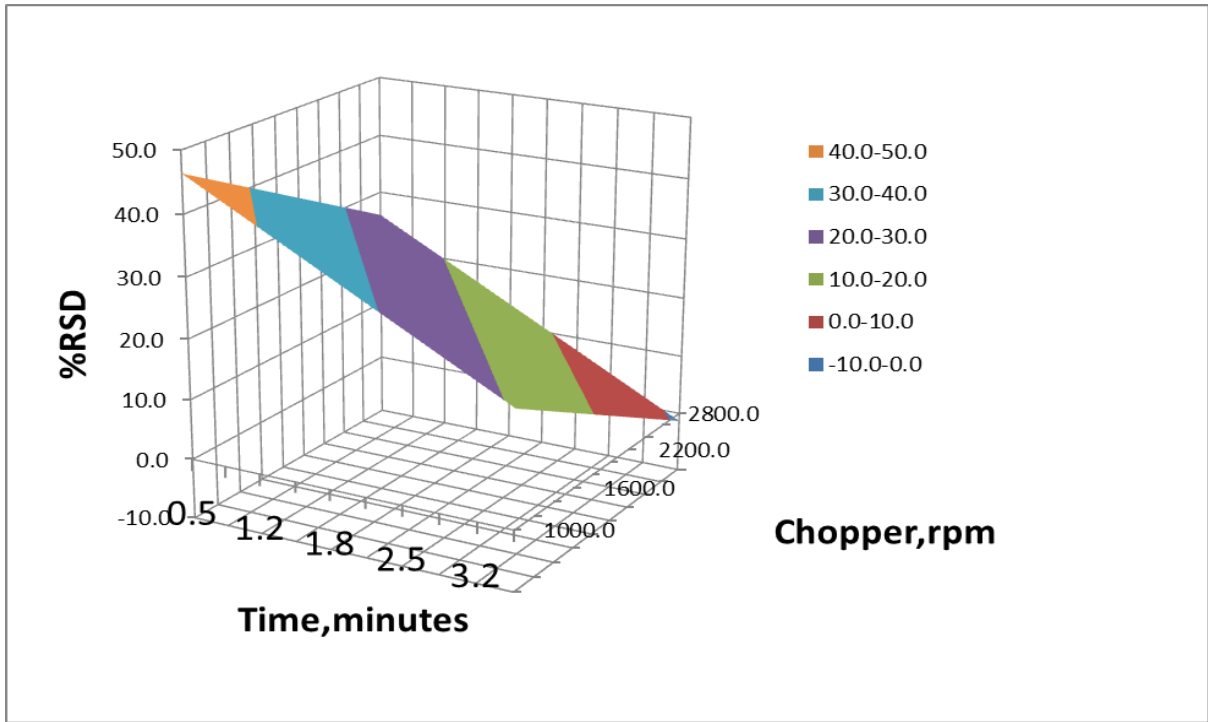


Figure 4.2: Response surface plot of estimated effects of mixing time and chopper blade speed

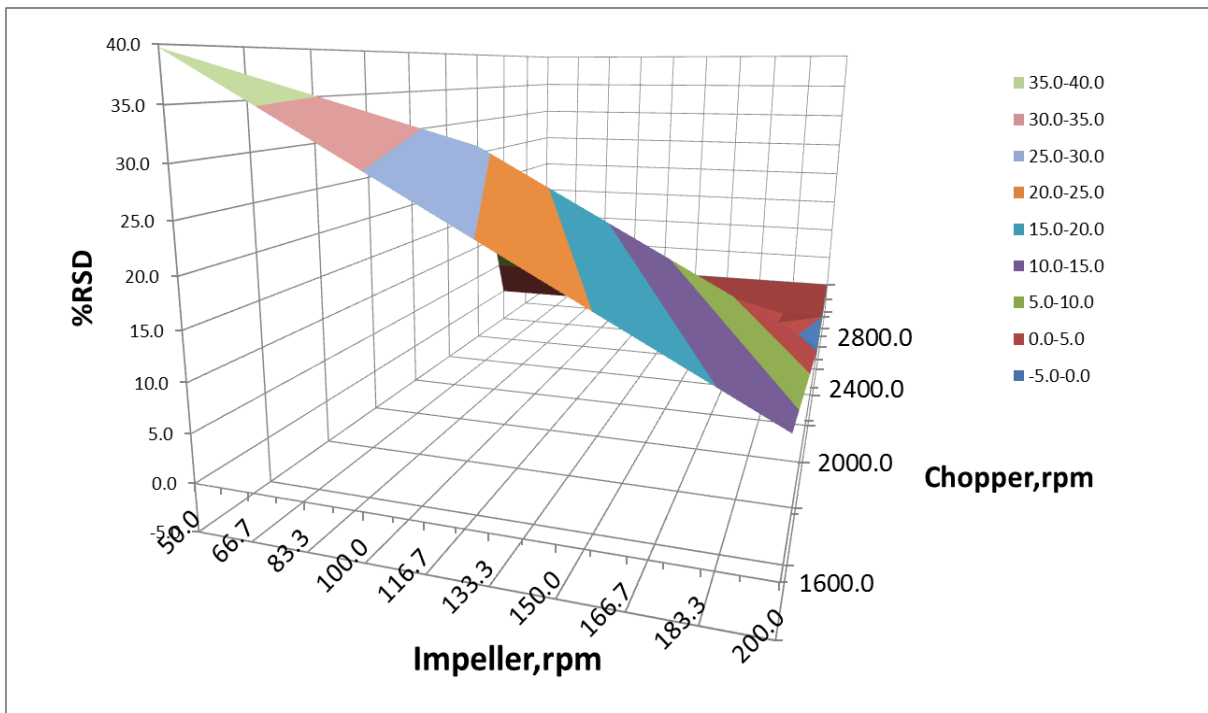


Figure 4.3: Response surface plot of estimated effects of chopper blade and impeller blade speeds

From figure 4.1 it is clear that as the mixing time and impeller blade speed is increased, the % RSD becomes smaller, which indicates a more homogenous mix is produced. When the mix is mixed for a longer time, the powders are mixed in the mixer bowl for longer, which produces a mix that is more homogenous. The samples tested from the bowl therefore have assay results with values close to each other. The % RSD for the seven samples taken from the bowl is hence lower. With an impeller blade speed that is higher, the development of slip planes within a bed of materials or the splitting of the bed of material to disintegrate agglomerates to overcome cohesion happens at a faster rate and therefore more slip planes are formed in a shorter amount of time which results in mixing of powders taking place faster. With a low impeller blade speed, the slip planes in the mix will form at a slower rate. It takes longer for the powders to be mixed homogeneously. The % RSD value in the plot decreases from 40.0 - 50.0 % RSD to -10.0 - 0.0 % with the impeller blade speed and mixing time at its highest values on the plot.

The % RSD is higher with impeller blade speed and mixing time at its lowest (figure 4.1) compared to the chopper blade and mixing time at its lowest settings (figure 4.2). This indicates that the mixer speed has a bigger impact on the homogeneity of the mix.

From figure 4.2 it is clear that as mixing time and chopper blade speed are increased, the % RSD becomes smaller, which is indicative of a more homogenous mix. This plot reiterates the data on mixing time in figure 4.1. With a higher chopper blade speed, agglomerates of powders are broken down at a faster rate and the powders can mix faster. The % RSD value in the plot decreases from 40.0 - 50.0 % RSD to -10.0 - 0.0 % with the chopper blade speed and mixing time at its highest values on the plot.

From figure 4.3 it is clear that as impeller blade and chopper blade speeds are increased, the % RSD becomes smaller, which is indicative of a more homogenous mix. The % RSD value in the plot decreases from 35.0 - 40.0 % RSD to -5.0 - 0.0 % with the chopper blade speed and impeller blade speed set at its highest values on the plot.

The % RSD value is the highest in figure 4.1, which may indicate that the combination of impeller speed and mixing time has the most significant impact on the % RSD result.

During the mixing process slip planes are formed within the bed of materials by the impeller blade and

agglomerates are disintegrated to overcome cohesion forces between particles. Agglomerates are broken down also by the chopper blade. Powders are hence mixed as it is moved through the mixing bowl.

4.1.3 Measures of model fit

The coefficient of determination (R^2): This value indicates how well the regression model fits the data. The R^2 adjusted value for the regression analysis was 0.687. This indicated that 68.7 % of variation in % RSD is explained by the regression model.

4.1.4 Model adequacy graphs

Figure 4.4 and table 4.6 depict the actual % RSD vs. the predicted % RSD (from the model). The residuals or errors were calculated from equations 7 and 8.

$$\text{Actual values} - \text{fitted model} = \text{residuals} \quad \text{Equation 7}$$

$$\% \text{RSD} - \text{fitted \% RSD (model)} = \text{residuals.} \quad \text{Equation 8}$$

This model indicates that the results for the predicted % RSD fit the actual % RSD data quite adequately.

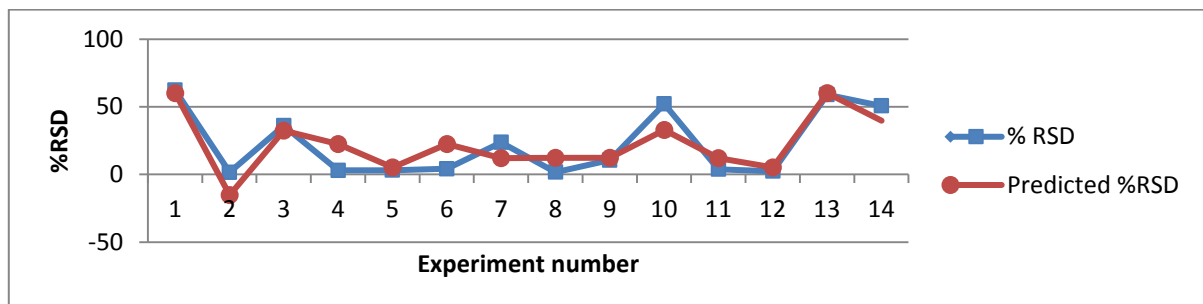


Figure 4.4: Comparison of the actual % RSD vs the predicted % RSD.

Table 4.6: Comparison of the actual % RSD vs the predicted % RSD with the 95 % confidence intervals

Exp #	%RSD	Predicted %RSD	+95 % Confid Int Pred	-95 % Confid Int Pred
1	62.33	60.20	94.89	25.51
2	1.71	-15.16	19.52	-49.85
3	36.04	32.57	67.25	-2.124
4	3.1	22.52	53.75	-8.719
5	3.19	5.257	39.95	-29.43
6	4.3	22.52	53.75	-8.719
7	23.77	12.14	46.83	-22.55
8	1.83	12.47	47.16	-22.22
9	10.7	12.47	47.16	-22.22
10	52.11	32.89	67.58	-1.800
11	3.86	12.14	46.83	-22.55
12	2.45	5.257	39.95	-29.43
13	59.06	60.20	94.89	25.51
14	50.78	39.78	74.46	5.086

The 95 % confidence interval prediction values indicate the % RSD results range for a specific experiment's operating parameters for which one could be 95 % certain that the predicted value would be accurate.

The residual values indicate the difference between the actual and predicted value.

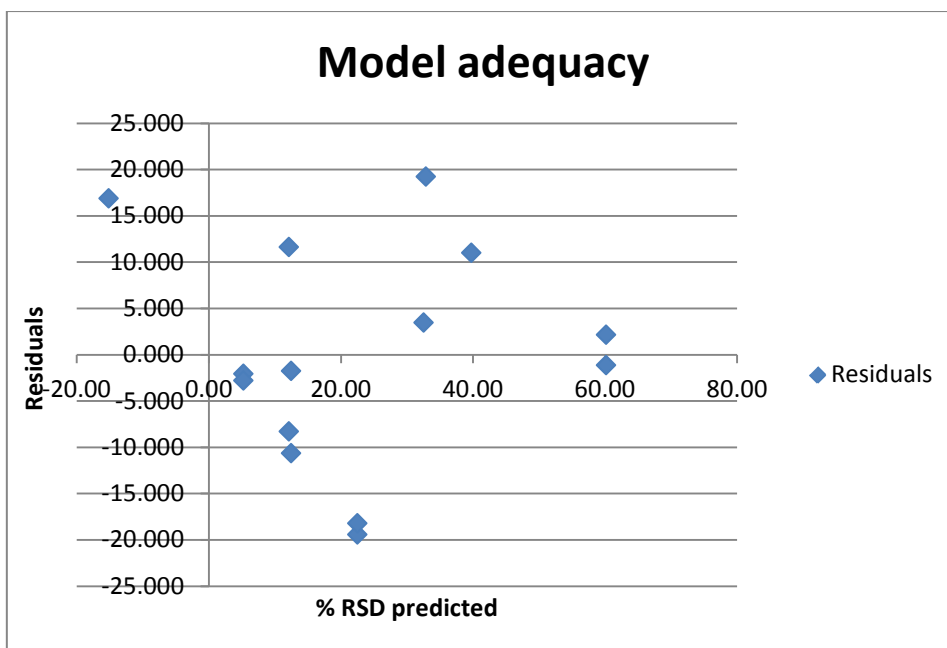


Figure 4.5: Model indicating the residuals

Table 4.7: Table indicating the actual % RSD vs the residuals

Exp #	% RSD	Residuals
1	62.33	2.133
2	1.71	16.87
3	36.04	3.474
4	3.1	-19.42
5	3.19	-2.067
6	4.3	-18.22
7	23.77	11.63
8	1.83	-10.64
9	10.7	-1.767
10	52.11	19.22
11	3.86	-8.284
12	2.45	-2.807
13	59.06	-1.137
14	50.78	11.00

4.1.5 Aliasing

Main effects are not aliased with each other but all main effects are aliased with all two way interactions.

4.2 Limitations

Problems were encountered with the model fit especially at 2 minutes and impeller blade speed set at 125 rpm, which is a limitation. The 2 minutes mixing time and impeller blade rpm could be increased in future studies in which a factorial design is used. This would then lead to a narrowing of the variation in the design of the experiment and allow the researcher to obtain actual values for the specific mixing equipment they are using.

The modelling in EREGRESS accounts for the experimental design. The problems with the converging with the fit are a limitation and the research is only exploratory to determine if the tested factors had an influence. Now that the influence of the various input factors is known, future research in the area of mixing of small amounts of API's could explore a larger range of formulations with a narrowing of the experimental values to obtain the actual quantitative effect on the setting for the operating parameters of the mixing equipment used.

5. DISCUSSION AND RECOMMENDATIONS

5.1 Discussion

The output (% RSD) from the different experiments amongst the three batches varied. The % standard deviation for the output of the three batches varied from 0.43 – 23.53 %. The result for % RSD obtained among the three batches were similar in most of the experiments indicating that the sampling method was also adequate. Experiments performed by Muzzio et al. (1999) showed that insertion of the side-sampling thief created significant disturbances in the mixture. Particles from upper layers were dragged deeply into lower layers as the thief penetrated the granular bed. The thief also had particles from all positions along the path of insertion and did not necessarily reflect the true composition of the system at the intended sampling location. As all the experiments for all three batches had been sampled the same way, this sampling method was adequate for the project. Future research could be performed on on-line technology – near infrared (NIR) – in the determination of homogeneity of dry powders during the dry mixing step in high shear mixer/granulators. The use of this technology has been explored in blenders. NIR can determine the homogeneity of dry powder blending online (Wechsler 2002).

The higher deviation among the results for the three batches was for the experiments where three or four of the parameters were at low settings (experiments 1, 3, 10, 13). The % RSD was above 10.00 %. The high variability for these experiments is due to inadequate mixing which results in high variability among the three batches.

For the other experiments the % standard deviation for the results obtained from the three batches were < 4.50 %.

The experiments where medium or high mixer blade speed and/or mixing time was used all passed the criteria of having a % RSD \leq 5 % for the average of the three batches (experiments 2, 4, 5, 6 and 12). For these experiments the results also pass the criteria of the 7 individual results being within 10 % of the average results. There are 2 experiments that have a % RSD of less than 5 % and have a low mixer speed or mixing time, but have a high concentration of enalapril. These are experiments 8 and 11. Experiment 10 had a very high % RSD and had the following settings: a low mixer speed,

high mixing time, but a low concentration. It is therefore clear that adequate mixing is easier to achieve when the drug loading is higher.

The combination of parameters used in experiments 1, 13 and 14 were inadequate and hardly any mixing took place. The mixing time and impeller blade speed were at the lower settings.

The % RSD values for the 2 centrepoinTs, which are experiments with the same operating parameters, were similar. The same operating parameters hence produced almost the same result when repeated.

From the plots in figures 4.1 to 4.3 and the results in table 4.2 it is clear that:

- (a) As the mixing time and impeller blade speed increases, the mixture becomes more even as the % RSD becomes lower. In figure 4.1, the % RSD goes into a negative. These two parameters were proven to have the biggest impact on the outcome of the efficiency of the mixture, as will be explained later.
- (b) As the mixing time and chopper blade speed increases, the mixture becomes more even as the % RSD becomes lower.
- (c) As the impeller and chopper blade speeds increase, the mixture becomes more even as the % RSD becomes lower. The lower the % RSD, the more even the distribution of the enalapril powder throughout the mix.
- (d) As the impeller blade speed increases, the mixture becomes more even as the % RSD becomes lower. This is applicable to low, medium and high percentages of enalapril in the mixture.
- (e) As the chopper blade speed increases, the mixture becomes more even as the % RSD becomes lower. This is especially evident in the experiments with a high percentage of enalapril in the mixture.
- (f) As the mixing time increases, the mixture becomes more even as the % RSD becomes lower. This is more evident in the experiments with high percentage of enalapril in the mixture. In the experiments with low percentage of enalapril in the mixture, it is clear that increasing the mixing time alone does not positively impact on the mix uniformity. In this case main impeller

blade and chopper blade speeds are also important, as the percentage of enalapril powder in the mixture is very low.

The Pearson correlation coefficient is equal to 89.8 % for the regression output model for all four parameters, which is close to a total positive correlation.

From the statistics in table 4.3, it can be seen that the p value for the chopper blade speed (0.02207), impeller blade speed (0.00465) and mixing time (0.00498) is less than 0.05, which makes these three parameters significant. The concentration is insignificant as the p value for this parameter is 0.174. The impeller blade speed and mixing time are the two parameters that are the most significant, followed by the chopper blade speed.

Backward elimination was performed and statistics were done for the three significant parameters.

From table 4.4 the following can be concluded:

The Pearson correlation coefficient is equal to 87.1 %. There is a good degree of linear dependence.

The p-value from the F-test is less than 0.05 ($F_{3,13}=10.50$; $p=0.00196$) indicating a significant fit of the model $\%RSD = 85.30 - 0.184 \cdot \text{Impeller} - 0.01135 \cdot \text{Chopper} - 9.103 \cdot \text{Time}$ at a 95% level of confidence.

The student t test and individual p values indicate the significance of the individual independent variables. The factor impeller blade has significant influence on % RSD at a 95% level of confidence with a p-value below 0.05 ($t=-3.534$; $p=0.00541$). The factor chopper also has significant influence on % RSD at a 95 % level of confidence with a p-value below 0.05 ($t=-2.612$; $p=0.02597$). The factor Time also has significant influence on % RSD at a 95 % level of confidence with a p-value below 0.05 ($t=-3.492$; $p=0.00580$).

There is a reasonable correlation between the actual and predicted values from the regression model ($R = 0.871$). The coefficient of determination (R^2) indicates how well the regression model fitted the data. In this case the R^2 value is 0.687. This indicated that 68.7 % of variation in % RSD is explained by the regression model. Please note that the adjusted R^2 value must be used if there is more than 1 independent variable as in this case.

The optimal parameters indicated are: impeller blade speed set at 191 rpm, chopper blade speed set at 2002 rpm and mixing time set at 3.01 minutes. The % RSD is very low at these parameters (0.007462 %).

The actual % RSD vs the predicted % RSD values are reasonably close, except for three of the experiments (4, 6 and 10). Experiments 4 and 6 had all the medium parameters and experiment 10 had low parameters for enalapril ratio, impeller blade speed and chopper blade speed.

The actual % RSD values obtained for the experiments and the predicted % RSD values are similar. The residuals for the actual vs. the predicted results are between 1.137 and 19.42. The model can hence predict optimal parameters with good confidence. In table 4.6 the range of % RSD values for which one could be 95 % confident that the value could be correct for a certain set of operating parameters is provided. For the parameters used in experiment 1 for example, if the % RSD falls between 25.51 and 94.89 one could be 95 % confident that that could be the value one would obtain for such experiment. From figure 4.5 it is evident that the residuals are lower for predicted %RSD values of between 0 and 10 % and between 50 and 70 %. In between these predicted %RSD values the residual is higher. This is preferred as one could be certain that the experiments which give very bad or very good mixing, can be predicted with more certainty as the residual between the actual and predicted values are lower. The predicted values will hence be close to the actual value.

Other approaches may have produced similar results. An experiment was conducted to compare a full factorial experiment with a Plackett-Burman design. For the full factorial design 32 experiments had to be performed compared to the 12 for the Plackett-Burman Design. The same factors showed up as significant. The full factorial design indicated the significant interactions whereas the Plackett-Burman design could not do that. The factor settings and conclusion remained the same when either design was used. There is however a significant difference in the number of experiments that needs to be conducted (Jayakumar 2013). For the purpose of this project, a Plackett-Burman design was sufficient as it provided a rough estimate of the main effects. Given the number of parameters and levels of these, a Plackett-Burman design was chosen for this project. With only a limited amount of experiments that had to be conducted, the necessary conclusions could be drawn.

5.2 Conclusions

Data on dry mixing in a high shear mixer is limited. This includes optimal parameters to be used as well as criteria for homogeneity.

By performing the dry mixing step and granulation in one piece of equipment saves time and therefore money. The equipment used is a high shear mixer instead of a blender for mixing and a high shear mixer for granulation.

As there are many parameters to be controlled during dry mixing in a high shear mixer, a statistical design method is suitable to establish the parameters that would have the most impact on the end result. After establishing this, further experimentation could be done to ensure that all the raw materials are evenly distributed at the dry mixing stage, ensuring the uniformity of the active pharmaceutical ingredient(s) and inactive pharmaceutical ingredient(s) in granules, before blending and compression. By using Design of Experiments, the industry could make more informed decisions when it comes to risk. By having a better understanding of the possible impact of, for example, a higher mixing speed and having results from this project to see the impact of a higher mixing speed, the risk to the final product of using a higher or lower mixing speed with the powders prior to granulation could be predicted with more confidence. These experimental results could be used during audits by external auditors as part of the data generated during the development of the product and to prove how the dry mixing parameters were established. Design of experiments is a tool that could be utilised much more during the development stage of products and during problem solving in production. The Plackett-Burman design is a tool that gives a lot of data with minimal experiments performed. The use of statistical design methods has demonstrated various advantages in previous studies. Four factors were investigated. These four factors were chosen based on literature data and experience.

Statistically it was found that mixing speed of the main impeller blade and chopper blade and overall mixing time are the three factors that have the biggest impact on the homogeneity of a mixture. These three parameters would thus be critical for process validation. The mixing time and impeller blade speed have proven to be more significant than the chopper blade speed.

Concentration was found to be insignificant and this is similar to the findings made by Kaufman in 1962 (active concentration did not affect the homogeneity of a mix), Venables & Wells in 2001 (drug content variation increases with decreasing drug content, but drug:excipient ratio has no effect) and Sudah et al. in 2002 (the concentration of the active component did not affect the outcome of the mixing process).

For our experiments and for the specific granulator used the following optimal parameters could be deduced: Impeller blade speed set at 191 rpm, chopper blade speed set at 2002 rpm and mixing time set at 3.01 minutes.

The experiments where medium or high mixer blade speed and/or mixing time were used also passed the criteria of having a % RSD \leq 5 % as well as individual results within 10 % of the mean result obtained.

I have thus achieved the aim of the study to determine the most significant parameters during mixing as well as optimal settings for dry mixing parameters in a high shear mixer/granulator.

Experimental design usage in pharmaceutical development, especially initial mixing, has great potential for further investigation to establish which are the factors for a specific active ingredient that have the biggest effect on optimal mixing.

5.3 Recommendations

Areas of possible future research that became evident through this study are:

- To explore the impact of moisture on the effectiveness of mixing.
- To explore the impact of different particle sizes of the Active Pharmaceutical Ingredient on the effectiveness of mixing using design of experiments.

- An evaluation of a number of other variables such as loading capacity, evaluation of other sample thief designs and extended mixing times on blend uniformity.
- Explore the possibility of using near infrared technologies for the determination of homogeneity of a dry mix in a high shear mixer/granulator.

6. REFERENCES

- Alayoubi, A., Nazzal, M., Sylvester, PW., Nazzal, A. 2013. "Vitamin E" fortified parenteral lipid emulsions: Plackett-Burman screening of primary process and composition parameters. *Drug Development and Industrial Pharmacy*. 39 (2): 363-373.
- Alexander, A., Sudah, O., Arratia, P., Duong, NH. 2004. Characterisation of the Performance of Bin Blenders: Part 3 of 3: Cohesive Powders. *Pharmaceutical Technology*. 28(9):54-74.
- Alexander, AW. & Muzzio, FJ. 2006. "Chapter 6: Batch Size Increase in Dry Blending and Mixing" in *Pharmaceutical Process Scale-up*, ed. Michael Levin, (USA: CRC Press), 2: 161-180.
- Armstrong, NA. 2006. Chapter 1: Introduction to Experimental Design in *Pharmaceutical Experimental Design and Interpretation*, (USA: CRC/Taylor & Francis), 2: 1.
- Barbosa-Cánovas, GV., Ortega-Rivas, E., Juliano, P., Yan, H. 2005. Chapter 9: Mixing in *Food engineering series*, ed. Gustavo V., (New York: Kluwer Academic/Plenum Publishers), 2: 221-244.
- Boatman, J. 2011. Presentation: The Incoming Bombshell: FDA's 2011 Process Validation Guidance.
- Bozzone, S. 2001. Solid Oral Dosage Forms Powder Blending. IKEV Meeting presentation 31 May 2001. Quality Operations Cork, Ireland.
- Bozzone, S. 2001. Solid Oral Dosage Forms Blend Uniformity: Principles and Examples. Turkish Pharmaceutical Society Meeting presentation 01 June 2001. Quality Operations Cork, Ireland.
- Buckley, D & Associates. 2004. The "Barr Laboratories" Court Decision United States District Court For the District of New Jersey Civil Action No. 92-1744. 17.
- Charoo, NA. & Ali, AA. 2013. Quality risk management in pharmaceutical development. *Drug Development and Industrial Pharmacy*, 39 (7): 947.
- DFE Pharma. 2013. Guide to tableting by wet granulation. Available online: www.dfepharma.com . (Downloaded November 09, 2013). Page 1-10.
- Du Toit, LC. 2007. Formulation of an anti-tuberculosis drug delivery system. *Masters dissertation*. Johannesburg, University of Witwatersrand: vi, 266.

Egermann H & Pichler E. 1988. Significance of drug content and of drug proportion to the content uniformity of solid dosage forms. *Acta Pharm, Jugoslavica*, 38 (4), 279-286.

Greaves, FC., Beasley, MW., Suddith, AW., Swarbrick, J.1995. Novel Approaches to the Preparation of Low-Dose Solid Dosage Forms. *Pharmaceutical Technology*, 01:60-64.

Gabrielsson, J., Sjöström, M., Lindberg, NO., Pihl, AC., Lundstedt, T. 2006. Robustness Testing of a Tablet Formulation Using Multivariate Design. *Drug Development and Industrial Pharmacy*, 32 (3): 297-307.

Gokhale, R, Sun, Y & Shukla, AJ. 2005. "Chapter 7: "High-Shear Granulation" in *Handbook of Pharmaceutical Granulation Technology*, ed. Dilip M. Parikh, (USA: Taylor & Francis Group), 2: 191.

Gokhale, R & Trivedi, NR. 2010. "Chapter 9: Wet Granulation in Low- and High-Shear Mixers" in *Handbook of Pharmaceutical Granulation Technology*, ed. Dilip M. Parikh, (USA: Taylor & Francis Group), 2: 183.

Goskonda, SR., Hileman, GA., Upadrashta, SM. 1994. Development of Matrix Controlled Release by Extrusion-Spheronization Technology Using Statistical Screening Design. *Drug Development and Industrial Pharmacy*, 20 (3): 279-292.

Harnby, NA. 1967. Comparison of the performance of industrial solids mixers using segregating material. *Powder Technology Journal*, 1: 94-102.

Hickey, AJ. & Ganderton, D. 2001. "Chapter 4: Powders" in *Pharmaceutical Process Engineering*, ed. James Swarbrick (New York: Marcel Dekker, Inc.), 112: 77.

Hileman, GA., Goskonda, SR., Spalitto, AJ., Upadrashta, SM. 1993. A Factorial Approach to High Dose Product Development by an Extrusion/Spheronization Process. *Drug Development and Industrial Pharmacy*, 19 (4): 483-491.

International Conference on Harmonisation. 2009. Pharmaceutical Development Q8 (R2).

International Conference on Harmonisation. 2008. Pharmaceutical Quality Systems Q10

International Conference on Harmonisation. 2005. Quality Risk Management Q9.

- Jacquez, JA. 1998. Design of Experiments. Pergamon, 3358 (2): 259-279.
- Jayakumar, M. 2013. When and how to use Plackett-Burman experimental design. Six sigma, www.isixsigma.com (accessed: 19.10.2014).
- Kapil, R., Dhawan, S., Beg, S., Singh, B. 2013. Buccoadhesive films for once-a-day administration of rivastigmine: systematic formulation development and pharmacokinetic evaluation. *Drug Development and Industrial Pharmacy*, 39 (3): 466-480.
- Kaufman, A. 1962. Mixing of solids, Experiments with Tumbling Blenders. I&EC Fundamentals, 1(2):104-106.
- Kenari, HS., Alinejad, Z., Imani, M., Nodehi, A. 2013. Effective parameters in determining cross-linked dextran microsphere characteristics: screening by Plackett-Burman design of experiments. *Journal of Microencapsulation*.
- Kiekens, F., Córdoba-Díaz, M. & Remon, JP. 1999. Influence of Chopper and Mixer Speeds and Microwave Powder Level During the High-Shear Granulation Process on the Final Granule Characteristics. *Drug Development and Industrial Pharmacy*, 25 (12): 1289-1293.
- Lewis, GA, Mathieu, D & Phan-Tan-Luu, R. 1999. *Pharmaceutical Experimental Design*, ed. James Swarbrick. (New York: Marcell Dekker, Inc.).
- Li, JZ., Rekhi, GS., Augsburger, LL., Shangraw, RF. 1996. The role of Intra- and Extragranular Microcrystalline Cellulose in Tablet Dissolution. *Pharmaceutical Development and Technology*, 1 (4): 343-355.
- Murphy, JR. 2010. Chapter 194: Screening Design. *Encyclopedia of Biopharmaceutical Statistics*, ed. Shein-Chung Chow, (USA: CRC, Taylor & Francis), 3.
- Muzzio, FJ., Roddy, M, Brone, D, Alexander, AW., Osama, S. 1999. An Improved Powder-Sampling Tool. *Pharmaceutical Technology*, 92-110.
- Myers, WR. 2010. Chapter 187: Response Surface Methodology. *Encyclopedia of Biopharmaceutical Statistics*, ed. Shein-Chung Chow, (USA: CRC, Taylor & Francis), 3.

Ngai, SSH. 2005. Multi-scale analysis and simulation of Powder Blending in Pharmaceutical Manufacturing. Massachusetts Institute of Technology Department of Chemical Engineering: Massachusetts PhD Thesis: 9-11, 17, 38.

Nutan, MTH., Vaithiyalingam, SR., Khan, MA. 2007. Controlled Release Multiparticulate Beads Coated with Starch Acetate: Material Characterization, and Identification of Critical Formulation and Process Variables. *Pharmaceutical Development and Technology*, 12 (3): 307-320.

Prescott, J & Garcia, T. 2001. Blending and Blend Uniformity. *Pharmaceutical Technology Journal*, 25 (3): 68-88.

Rambali, B., Baert, L., Thoné, D., Massart, DL. 2001. Using Experimental Design to Optimize the Process Parameters in Fluidized Bed Granulation. *Drug Development and Industrial Pharmacy*, 27(1): 47-55.

Sampling Systems. 2002-2013. Multi Thief. Available online:

http://www.sampling.co.uk/news_1006.html. (Downloaded: June 22, 2013).

Saral Engineering Company. Machines for solid dosage. Available online:

<http://saraleng.com/rmwgrnd.htm>. (Downloaded: June 22, 2013).

Saral. 2006. Installation, Operation & Maintenance Manual for Rapid Mixer and Wet Granulator 10 kg capacity. India.

Shah, RB., Nutan, M;.,Reddy, IK., Khan, MA. 2004. A Dual Controlled Gastrointestinal Therapeutic System of Salmon Calcitonin II. Screening of Process and Formulation Variables. *Clinical Research and Regulatory Affairs*, 21(3-4): 231-238.

Shargel, L. & Kanfer, I. 2005. *Generic Drug Product Development Solid Oral Dosage Forms*, (New York, USA: Marcel Dekker, Inc.).

Singh, B., Kapil, R., Nandi, M., Ahuja, N. 2011. Developing oral drug delivery systems using formulation by design: vital precepts, retrospect and prospects. *Expert opinion on Drug Delivery*, 8 (10): 1341-1360.

Skultety, PF., Rivera, D., Dunleavy, J., Lin, CT. 1988. Quantitation of the Amount and Uniformity of Aqueous Film Coating Applied to Tablets in a 24" Accela-Cota. *Drug Development and Industrial Pharmacy*, 14 (5): 617-631.

Skultety, PF., Sims, SM. 1987. Evaluation of the Loss of Propylene Glycol During Aqueous Film Coating. *Drug Development and Industrial Pharmacy*, 13 (12): 2209-2219.

Sokal, RR. & Rohlf, FJ. 1987. *Introduction to Biostatistics*. (USA: WH Freeman and Company), 2.

Sudah, OS., Arratia, PE., Coffin-Beach, D., Muzzio, FJ. 2002. Mixing of Cohesive Pharmaceutical Formulations in Tote (Bin) Blenders. *Drug Development and Industrial Pharmacy*, 28(8): 905-918.

Swaminathan V & Kildsig DO. 2002. Polydisperse Powder Mixtures: Effect of Particle Size and Shape on Mixture Stability. *Drug Development and Industrial Pharmacy*, 28(1): 41-48.

Tousey, MD. 2002. The granulation process 101. *Pharmaceutical Technology*, 8.

United States of America, Plaintiff, vs. Barr Laboratories, Inc. et al., Defendants. 30 March 1993. CIV. A. No. 92-1744 (United States District Court, D. New Jersey).

U.S. Food and Drug Administration. 2014. Code of Federal Regulations Title 21 (Chapter 1 Food and Drug Administration Department of Health and Human Services Subchapter C-Drugs:General) Part 211 (Current Good Manufacturing Practice for Finished Pharmaceuticals) Subpart J (records and reports) Section 211.194 (Laboratory records) (a)(2).

US Food and Drug Administration (FDA). Revised as of April 1, 2013. Code of Federal Regulations Title 21 (Food and Drugs Chapter I Food and Drugs Administration Department of Health and Human Services Subchapter C-Drugs:General) Part 211 (Current Good Manufacturing Practice for Finished Pharmaceuticals) Subpart F (Production and Process Controls) Section 110 (Sampling and testing of in-process materials and drug products) (a) (3). Online access: <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcr/CFRSearch.cfm?fr=211.110>. Date accessed: 06 March 2014.

US Food and Drug Administration (FDA). 2003. Guidance for Industry: Powder Blends and Finished Dosage Units – Stratified In-Process Dosage Unit Sampling and Assessment. 7.

US Department of Health and Human Services. 2011. Guidance for Industry, Process Validation: General Principles and Practices, Revision 1: 1, 4, 8, 9.

US Pharmacopoeia. 2011. Official monograph: enalapril maleate tablets.

Vanaja, K., Shobha Rani, RH. 2007. Design of Experiments: Concepts and Applications of Plackett-Burman Designs. *Clinical Research and Regulatory Affairs*, 24 (1): 1-23.

Venables, HJ. and Wells, JI. 2001. Powder Mixing. *Drug Development and Industrial Pharmacy*, 27(7): 599-612.

Vojnovic, D, Selenati, P, Rubessa, F, Moneghini, M & Zanchetta, A. 1992. Wet granulation in a small scale high shear mixer. *Drug Development and Industrial Pharmacy*, 18 (9): 961-972.

Vora, N and Rana, V. 2008. Preparation and Optimization of Mouth/Orally Dissolving Tablets Using Combination of Glycine, Carboxymethyl Cellulose and Sodium Alginate: A Comparison with Superdisintegrants. *Pharmaceutical Development and Technology*. 13 (3): 233-243.

Walliman, N. 2005. *Your Research Project*. Great Britian: Sage Publications, 2.

Wechsler, J. 2002, Modernizing pharmaceutical manufacturing. *Pharmaceutical Technology*, 26 (2):16-24.

Wytenbach, N., Birringer, C., Alsenz, J., Kuentz, M. 2005. Drug-Excipient Compatibility Testing Using a High-Throughput Approach and Statistical Design. *Pharmaceutical Development and Technology*, 10 (4): 499-505.