ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 16, Issue, 04, pp. 27787-27791, April, 2024
DOI: https://doi.org/10.24941/ijcr.47034.04.2024

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

ASSESSMENT OF PROCESS VALIDATION PROGRAM IN BATCH PRODUCTION OF STRESOMIX PREMIX™: A FOCUS ON OPERATIONAL QUALIFICATION PROCEDURES WITHIN THE HERBAL INDUSTRY

Sharma Anirudh, Lata Pushap, Bisht Alpna and *Thakur Deepak

R&D Centre, Ayurvet Limited, Village Katha, P.O. Baddi – 173205, District Solan, Himachal Pradesh, India.

ARTICLE INFO

Article History:
Received 20th January, 2024
Received in revised form
19th February, 2024
Accepted 15th March, 2024
Published online 17th April, 2024

Key words:
Blending, Powder, Process validation, RP-HPLC-PDA, Stresomix Premix™

***Corresponding author:**
Thakur Deepak

ABSTRACT

Regulatory bodies throughout the world have ensured that formulations must be manufactured to the highest quality levels. The Food and Drug Administration (FDA) has a large-scale development program that is currently testing continuous production processes. Powder mixing is an important operation routinely used in many industries, including herbal medicine industry. The PAT (Process Analytical Technology) initiative requires the implementation of process validation, including in-process monitoring systems and controls, in the blending or mixing process. The quality of products depends on certain operating conditions, such as equipment, technical parameters, and formulation. In this work, an operational qualification for the blending process in the manufacturing of Stresomix Premix™ during large-scale production was performed. Blender operation was tested to illustrate the effect of mixing time on the homogeneity of the phytoconstituents in the herbal formulation, demonstrating that this parameter can be used as a secure parameter to control this process. A simple and practical operational qualification procedure has been proposed to investigate the blending operation on a large-scale production of Stresomix Premix™. Using an authentic analytical method that reliably profiles the phytoactives helped in validating the manufacturing process. The effects of mixing time have been quantified, and the behaviour of the dry powder mass was evaluated. A relation between theoretical concepts and real conditions that are applied in routine industrial production allowed the association of the critical operational aspects with the practical effects. The observed results illustrate that the homogeneity of powder in the blender depends on mixing time; it is important to consider this parameter to avoid segregation, mainly in formulations with a more complex composition and elevated powder mass. Additionally, results of this study demonstrates that the manufacturing process stands validated as it met acceptance criteria.

Citation: ***Sharma Anirudh, Lata Pushap, Bisht Alpna, Thakur Deepak*, 2024.** "Assessment of process validation program in batch production of stresomix premix™: a focus on operational qualification procedures within the herbal industry.*". International Journal of Current Research*, 16, (04), 27787-27791.

INTRODUCTION

Powdered herb blending is a prevalent practice within the herbal industry and stands as a pivotal unit operation in the manufacturing process of solid dosage forms. Product quality hinges on the thoroughness of blending its constituent materials, ensuring the uniformity of the end product. Moreover, the herbal medicinal sector places significant emphasis on powder blend content consistency, given that most products available are solid dosage forms. As numerous factors can influence blending quality, it becomes imperative to investigate them thoroughly during both product development and production phases. In addition to the physical attributes of powders like description, particle density, and surface properties, ensuring the uniformity of phytoconstituents is crucial, primarily to assess the likelihood of mixture segregation. Factors pertaining to equipment, operation, and formulation, including mixer design and operation, a combination of operational parameters, and mixture composition, also merit careful consideration. Within this framework, optimizing production operations involves implementing concepts, techniques, and traceability requirements.[1-2] The procedure for formulating a herbal premix involves a sequence of unit operations, depicted by a flow diagram comprising logically defined steps or modules, culminating in the production of the final herbal product. Critical unit operations are identified by analysing process variables and their corresponding measured responses. In the blending operation, variables such as load, speed, and mixing time are pivotal, given that achieving blending uniformity is the primary objective. Numerous studies have explored technologies for assessing blend homogeneity and determining the optimal blending duration.

In large-scale pharmaceutical production or during the scaling-up process, it is imperative to establish operational parameters for all steps, as mandated by regulatory requirements and included in process validation documents.[3-4] Understanding content uniformity in powdered mixtures is vital and considered a critical attribute to guarantee the quality of the final product. Therefore, industries must test optimal conditions to acquire reliable data for the final product. This necessity has led to the integration of a process analytical technology (PAT) system, aimed at developing efficient tools for pharmaceutical development, manufacturing, and product quality assurance.[5] In this paper, we present and deliberate on the findings from experiments that examine blending as an integral aspect of the process validation for the standard polyherbal formulation Stresomix Premix™, conducted on a large scale. We particularly emphasize the impact of mixing time and its correlation with key theoretical concepts. Stresomix Premix™ represents a proprietary polyherbal formulation developed by Ayurvet Limited, a wholly owned subsidiary of Zenex Animal Health India Private Ltd., specifically tailored for livestock animals. This clinically validated herbal blend aims to bolster the immune system function and aid in adaptation. Notably, Stresomix Premix™ powder is effective in combating heat stress while concurrently enhancing production, performance, and meat quality in livestock.

The formulation comprises a scientific amalgamation of various herbs, with its clinical efficacy stemming from the synergistic actions of its constituent herbs, thereby facilitating improved and expedited effects. Key ingredients within Stresomix Premix™ include *Ocimum sanctum, Withania somnifera, Phyllanthus emblica, Asparagus racemosus, Glycyrrhiza glabra, Tribulus terrestris, Mangifera indica, and Shilajit.*[6-10] As part of the prerequisites for manufacturing process validation, the protocol was devised to validate the manufacturing process of the product under investigation. Prior to commencing the process validation study, environmental conditions were evaluated against predefined acceptance criteria. Subsequently, a meticulously designed sampling plan delineating all locations and time intervals for sample collection was established, and sampling was executed accordingly. A total of 81 samples were gathered from various positions within the blender at intervals of 15, 30, and 45 minutes.[11-12]

To assess the active content in the samples, an analytical method was developed as an integral component of the exercise in the Research and Development (R&D) department. Gallic acid, an active biomarker (Figure 1), was chosen in relation to the herb (*Phyllanthus emblica*) incorporated in the formulation. The method underwent validation based on criteria including selectivity, linearity, precision, accuracy, limit of detection, and limit of quantification, in accordance with the International Conference on Harmonization (ICH) guidelines.[13] The estimation of % active content Gallic acid was conducted following its validated analytical method. The process was deemed validated if the percentage coefficient of variance (CV) was observed to be not more than 8% between the two extremes of percentage active content obtained after analysis.

EXPERIMENTAL

Reagents and materials: All chemicals and solvents utilized were of analytical or HPLC grade and sourced from E Merck,

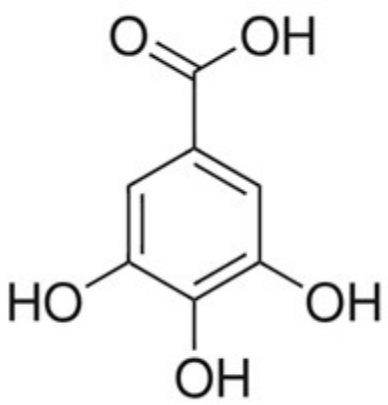

Figure 1. Gallic acid

Rankem, and SD Fine. The bioactive marker, i.e., Gallic acid, was procured from HIMEDIA. Samples of Stresomix Premix™ were acquired from the QA/QC department of Ayurvet Limited, a wholly owned subsidiary of Zenex Animal Health India Private Ltd., based in Baddi.

Blending and sampling experiments: The powder underwent blending in a stainless-steel Ribbon blender operating at a rotational speed of 25 rpm. During the batch processing of 2000 Kg, sampling was conducted at 15, 30, and 45-minute intervals. At each interval, the ribbon blender was halted, and samples weighing approximately 50.0 g were retrieved using a thief probe from nine positions within the blender (Top left, Middle left, Bottom left, Top center, Middle center, Bottom center, Top right, Middle right, Bottom right) in triplicates.

Homogeneity assessment and RP-HPLC-PDA measurements: Homogeneity assessment was conducted to evaluate the effectiveness of the mixing process. The quantity of Gallic acid, serving as a bioactive marker compound, was determined from each collected sample at various time points.

Standard Gallic Acid Preparation: Approximately 2.5 mg of standard Gallic acid was precisely weighed and dissolved in 50 mL of methanol to achieve stock concentrations of 50 µg/mL. The stock solutions were further diluted to establish a dilution range of 5 - 45 µg/mL and subsequently injected into the HPLC system to construct calibration graphs and quantify the bioactive compound.

Test Solution Preparation: For the quantification of Gallic acid, 5 g of Stresomix Premix™ underwent treatment with 70 mL of methanol under reflux conditions for 1 hour, followed by filtration. This process was repeated twice. The final volume was adjusted to 200 mL with methanol. Subsequently, 1 mL of this stock solution was diluted to 10 mL using the same solvent. Before injection into the HPLC system, the solution was filtered through a 0.45 µm membrane filter.

High Performance Liquid Chromatography

Instrumentation and Operating Conditions: The analysis of Gallic acid content was performed using Reverse Phase-High Performance Liquid Chromatography (WATERS, equipped with a binary pump-515 and PDA-2996 detector, USA). Data acquisition was facilitated through Empower 2.0 controlling software. Separation was achieved using a Phenomenex Luna C-18 column (250 mm x 4.6 mm, 5µm).

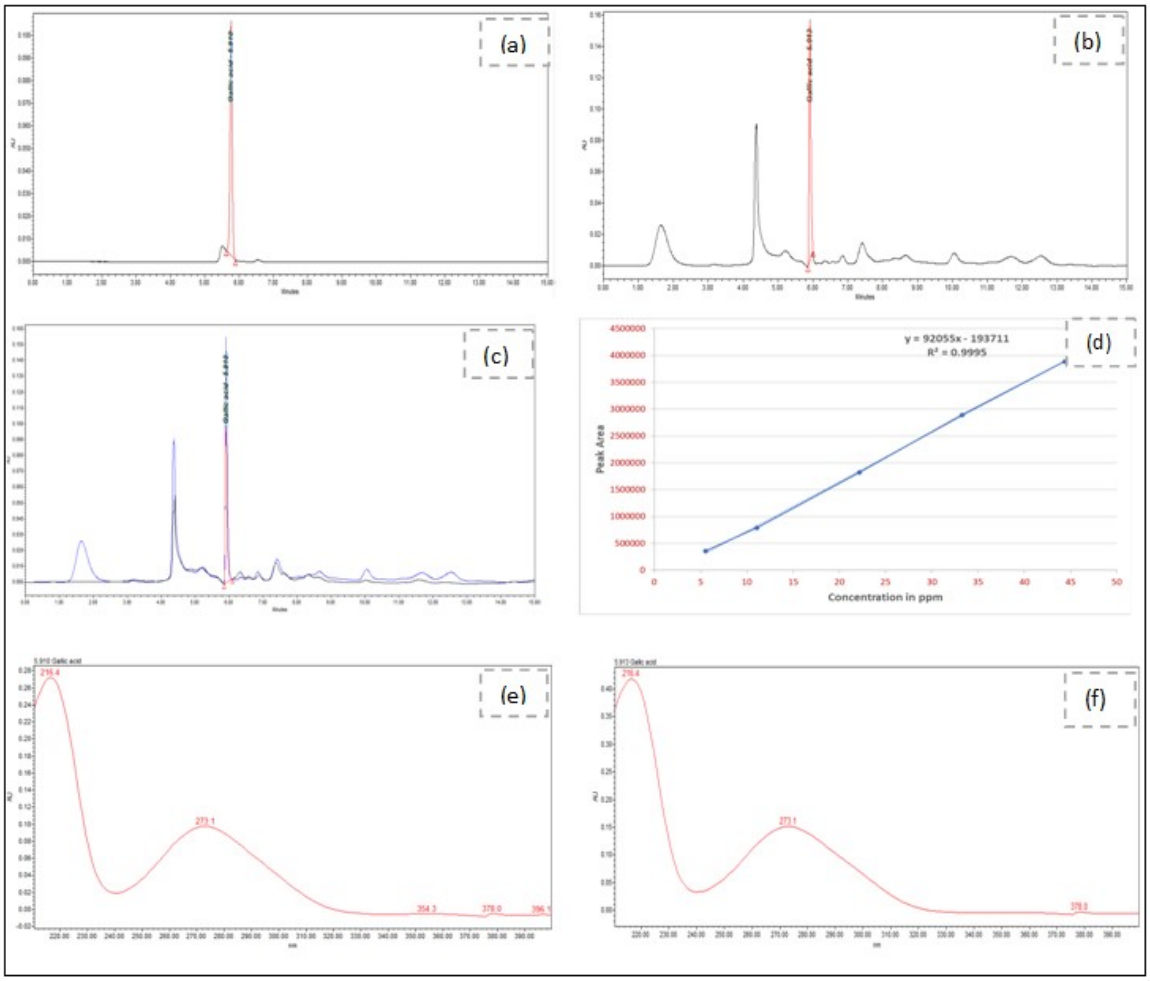

Figure 2. Chromatograms showing the resolution of marker compound in the formulation. (a) Chromatogram of standard Gallic acid. (b) Chromatogram of sample Stresomix Premix™. (c) Overlay of the Gallic acid chromatograms i.e. sample against standard. (d) Calibration plot for Gallic acid standard. (e) Spectral scan of standard Gallic acid standard. (f) Spectral scan of Gallic acid in Stresomix Premix™

Selection and Optimization of Chromatographic Conditions: Various mobile phase compositions were explored to optimize the RP-HPLC parameters. Satisfactory separation and peak symmetry for Gallic acid (Figure 1) were attained using a mobile phase consisting of Water : Acetonitrile in an 80:20, v/v ratio, with a pH of 3.0 adjusted using orthophosphoric acid, in isocratic mode. Prior to use, the mobile phase underwent filtration through a 0.45 µm Millipore filter and degassing. The flow rate was set at 0.7 mL/min. Injection volume was adjusted to 20 µL, and detection was conducted at a wavelength of 272 nm.

Method Validation: The analytical results obtained from the developed method are deemed valid only if the specified system suitability criteria are met. In this study, the experimental findings (Table 1) indicate that the chromatographic system was suitable for the intended analysis. A standard solution mixture containing a known concentration of Gallic acid was separately injected six times. The relative standard deviation (RSD) values for peak area and retention time of the standard solution suggested reproducibility for these parameters. The low RSD values observed for the tailing factor and theoretical plates implied good peak symmetry of Gallic acid and efficient column performance. Additionally, the proposed method underwent validation for the determination of Gallic acid using parameters such as calibration, precision, limit of detection (LOD), limit of quantification (LOQ), selectivity, and accuracy, in accordance with ICH guidelines (Table 2 & Figure 2).[14]

Table 1. Chromatographic parameters

S. no.	Parameter	Data	RSD
1	Peak area	453112	0.18
2	Retention time (minutes)	5.77	0.21
3	Theoretical Plates	11458	0.56
4	Tailing factor	0.988	0.84

Table 2. Results of precision, linear regression analysis and their corelation coefficient for quantitative analysis of marker compound

S. no.	Parameter	Gallic acid
1	Concentration range for linearity (*µg/mL*)	5 - 45
2	Correlation coefficient (r^2)	0.9995
3	Amount of marker compound in Stresomix Premix™ *(% w/w)*	0.73
4	Method precision (*repeatability n=7*) – RSD %	1.08
5	Intermediate precision (Reproducibility)- RSD %	1.07
	Intra-day	1.15
	Inter-day	
6	LOD (*µg/mL*)	0.292
7	LOQ (*µg/mL*)	0.972
8	Recovery studies *(n=3)* (%)	96.12

LOD: Limit of detection, LOQ: Limit of quantification, RSD: Relative standard deviation.

Table 3. Gallic acid content in Stresomix premix™

S. no.	Time of sampling	Repetitions	% w/w of Gallic acid content in Stresomix premix™								
			TR	TC	TL	MR	MC	ML	BR	BC	BL
1	15 min	A	0.80	0.82	0.76	0.84	0.78	0.85	0.84	0.76	0.87
		B	0.60	0.75	0.75	0.77	0.60	0.62	0.76	0.76	0.71
		C	0.59	0.61	0.61	0.60	0.60	0.61	0.62	0.59	0.56
		Mean	0.66	0.72	0.71	0.73	0.66	0.69	0.74	0.7	0.71
		CV	0.0401								
		% CV	4.01								
2	30 min	A	0.83	0.81	0.82	0.66	0.84	0.87	0.82	0.84	0.67
		B	0.81	0.85	0.86	0.82	0.66	0.83	0.91	0.83	0.90
		C	0.64	0.65	0.67	0.66	0.65	0.66	0.65	0.68	0.68
		Mean	0.76	0.77	0.78	0.71	0.72	0.79	0.79	0.78	0.75
		CV	0.0386								
		% CV	3.86								
3	45 min	A	0.72	0.69	0.64	0.69	0.71	0.73	0.7	0.64	0.63
		B	0.71	0.54	0.65	0.55	0.71	0.71	0.56	0.63	0.72
		C	0.55	0.69	0.66	0.55	0.71	0.56	0.70	0.77	0.76
		Mean	0.66	0.64	0.65	0.6	0.71	0.66	0.65	0.68	0.70
		CV	0.0498								
		% CV	4.98								

Where TR = Top Right; TC= Top Center; TL=Top Left; MR = Medium Right; MC= Medium Center; ML= Medium Left; BR = Bottom Right; BC= Bottom Center; BL= Bottom Left.

RESULTS AND DISCUSSION

In the context of the Stresomix Premix™ powder mixing operation, which involves various polyherbal excipients in varying quantities, effective performance relies on several factors including equipment, mixing parameters (rotation speed, mixing time, and vessel filling level), and formulation. To enhance mixing control, Process Analytical Technology (PAT) has been developed. PAT facilitates the design, analysis, and control of manufacturing processes through real-time measurements of critical quality and performance attributes of raw and in-process materials. The manufacturing process of Stresomix Premix™ underwent validation regarding blending time to ensure consistent product quality and to determine the optimal duration necessary to achieve it. Samples were collected according to the sampling plan, analysed for Gallic acid content using RP-HPLC, and yielded results ranging from 0.55% to 0.91% (Table 3). During the manufacturing process, the % CV (percent coefficient of variance) ranged from 3.86 to 4.98 across the 15, 30, and 45-minute mixing intervals. The minimum % CV of 3.86 was attained well within the 30-minute mixing duration, meeting the criteria for fair mixing as per standard norms and procedures applicable to the blending of any specific formulation, wherein the % CV upper limit is stipulated as 8.0. During solid dosage forms production, meticulous control of various process steps is essential, and such controls must be conducted regularly. Across industries worldwide, blending or mixing operations are integral at various stages of manufacturing. In pharmacy, the primary goal of mixing is to achieve dosage units that contain uniform quantities of drugs. This objective necessitates obtaining a homogeneous blend of multiple solid products. Achieving homogeneity requires consideration of raw material characteristics, the final mixture, equipment specifications, and operating conditions. Within the pharmaceutical industry, process validation emerges as a pivotal and mandatory tool in accordance with regulatory mandates. As defined by the FDA, process validation encompasses the collection and evaluation of data from the process design phase through production, establishing scientific evidence of consistent product quality. The validation process spans the product and process lifecycle, encompassing process design, qualification, and ongoing verification.[15] During the process qualification phase, the sampling plan incorporates sampling points, sample quantities, and sampling frequency for each unit operation. The selection criteria hinge upon material characteristics such as density, particle size, and component quantities. Failure to tightly control these factors may jeopardize the final product's quality. The implementation of PAT necessitates in-process monitoring systems and controls within the mixing process. Various technologies, including RP-HPLC-PDA, HPTLC, NIR spectroscopy, magnetic resonance imaging, electrical capacitance, and visual observation, are proposed for in-line monitoring of content uniformity. These techniques prove particularly valuable for continuous blending processes in large-scale production scenarios.

CONCLUSION

A simple and practical operational qualification procedure has been proposed to investigate the herbal product blending operation on a large scale. The effects of mixing time have been quantified and the behaviour of dry powder mass was evaluated, considering the equipment (ribbon blender). A relation between theoretical concepts and real conditions that are applied in routine industrial production allowed the association of the critical operational aspects with the practical effects. In the current study, manufacturing process stands validated as it met acceptance criteria and 30 minutes was concluded to be optimal mixing time for uniformity of product's active ingredients. The observed results illustrate that the homogeneity of powder into the blender depends on mixing time; it is important to consider this parameter to avoid segregation, mainly in formulations with more complex herbal composition and with large batch size.

DECLARATIONS

Funding: None.

Conflict of Interest: None declared.

Ethical approval: Not required.

REFERENCES

1. Cleary PW, Sinnott MD. Assessing mixing characteristics of particle-mixing and granulation devices. Particuology. 2008 Dec 1;6(6):419-44.
2. Sarkar A, Wassgren CR. Simulation of a continuous granular mixer: Effect of operating conditions on flow and mixing. Chemical Engineering Science. 2009 Jun 1;64(11):2672-82.
3. Santomaso A, Olivi M, Canu P. Mechanisms of mixing of granular materials in drum mixers under rolling regime. Chemical Engineering Science. 2004 Aug 1;59(16):3269-80.
4. Léonard G, Bertrand F, Chaouki J, Gosselin PM. An experimental investigation of effusivity as an indicator of powder blend uniformity. Powder Technology. 2008 Feb 6;181(2):149-59.
5. Food and Drug Administration. Guidance for industry, PAT-A framework for innovative pharmaceutical development, manufacturing and quality assurance. http://www. fda. gov/cder/guidance/published. html. 2004.
6. Modzelewska-Kapituła M, Tkacz K, Nogalski Z, Karpińska-Tymoszczyk M, Draszanowska A, Pietrzak-Fiećko R, Purwin C, Lipiński K. Addition of herbal extracts to the Holstein-Friesian bulls' diet changes the quality of beef. Meat science. 2018 Nov 1;145:163-70.
7. Hemanth G Keregallikoppalu*, Madhusudhan HS, Ravikumar Puttamallappa and Naveenkumar GS. "Heat Ameliorating Activity of Herbal Antistress Formulation in Broiler under Heat Stress". International Conference on Biodiversity, Climate Change Assessment and Impacts on Livelihood, 10-12 January, 2017, Kathmandu, Nepal.
8. Giannenas I, Bonos E, Skoufos I, Tzora A, Stylianaki I, Lazari D, Tsinas A, Christaki E, Florou-Paneri P. Effect of herbal feed additives on performance parameters, intestinal microbiota, intestinal morphology and meat lipid oxidation of broiler chickens. British Poultry Science. 2018 Sep 3;59(5):545-53.
9. Ilias G. et.al. "Effect of dietary supplementation with a plant extract mix on growth performance, intestinal microbiota and morphometry of broiler chickens." PanHellenic Congress in technology of Animal Production, Thessalaniki, Greece, 3 Feb-2017.
10. Ilias G. et.al." Effect of dietary supplementation of herbal products on broiler growth performance, gut health and meat quality" World Veterinary Poultry Association XXth Congress 2017, Edinburgh 4 - 8 September 2017.
11. Kotagiri Ravikanth, Anirudh Sharma, Deepak Thakur and Pushap Lata. "Quality assertiveness by industrial process validation program of superliv ds premix formulation", International Journal of Current Research. 2020;12, (01), 9217-9223.
12. Kotagiri Ravikanth, Kanaujia Anil, Thakur Deepak, et.al. "Quality Assurance by Effective Manufacturing Process Validation". International Journal of Pharmaceutical Quality Assurance 2015; 6(4): 109-113.
13. Guidance for Industry: Process Validation: General Principles and Practices. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), Center for Veterinary Medicine (CVM), November 2011.
14. ICH harmonized tripartite guideline, Q8 Guidelines for Pharmaceutical Development, Revision 2, Geneva, Switzerland; 2009; 12-14.
15. FDA, Food and Drug Administration. Process Validation. General Principles and Practices, U.S. Department of Health and Human Services, 2008.

****