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Research Article

Quality Assurance by Effective Manufacturing Process Validation

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ABSTRACT

Validation study is the core subject of study in quality assurance field and effective process validation as a part of QbD contributes significantly to assuring product quality. As per ICH Q8 guidelines of pharmaceutical development a critical quality attribute is a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality. The enormous growth of herbal medicinal products worldwide has been one of the most interesting aspects of healthcare. As pharmacological properties of an herbal formulation depend on phytochemical constituents present therein, it becomes inevitable to incorporate quality by design concept in manufacturing processes to ensure predefined product specifications are met. Development of authentic analytical methods which can reliably profile the phytochemical composition and help in validation of manufacturing process is a major challenge to scientists. Prior standardization of formulation during its designing and development stage with respect to its bioactive marker compounds as a key feature of CQA (critical quality attribute) ensures the phytoequivalence during the manufacturing of product on commercial scale. This will ensure the batch to batch consistency in quality & efficacy.

INTRODUCTION

For a high quality drug product it is important that quality should be built in by design thus ensuring the quality from its very inception. A key tenet of Qbd (Quality by Design) approach is systematic evaluation of quality and procedures at every step of the process, with an eye towards quick identification and rectifications of shortfalls. It ensures that a system is in place to characterize the raw materials and control for quality at several stages of the cycle. The application of concept provides a higher level of assurance of drug product quality & cost savings.

Validation study is the core subject of study in quality assurance field and effective process validation as a part of QbD contributes significantly to assuring product quality. To carry out process validation, it is essential to determine critical quality attributes and control variables. As per ICH Q8 guidelines of pharmaceutical development a critical quality attribute is a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality. The list of potential CQAs can be modified when the formulation and manufacturing process are selected and as product knowledge and process understanding increase. Quality risk management can be used to prioritize the list of potential CQAs for subsequent evaluation (1).

The enormous growth of herbal medicinal products worldwide has been one of the most interesting aspects of healthcare. Harmonization on the different facets of development of herbal medicines, including their quality, safety, efficacy, validation and regulation is imperative to create the better image of herbal drug industry across the globe. As pharmacological properties of an herbal formulation depend on phytochemical constituents present therein, it becomes inevitable to incorporate quality by design concept in manufacturing processes to ensure predefined product specifications are met. Development of authentic analytical methods which can reliably profile the phytochemical composition and help in validation of manufacturing process is a major challenge to scientists. Prior standardization of formulation during its designing and development stage with respect to its bioactive marker compounds as a key feature of CQA (critical quality attribute) ensures the phytoequivalence during the manufacturing of product on commercial scale. This will ensure the batch to batch consistency in quality & efficacy.

Manufacturing process of Pulmofarm T, herbal premix to relieve respiratory distress and a proprietary product of AYURVET LIMITED was undertaken for its validation as part of QbD in herbal products manufacturing process.

The herbal premix was designed and developed at our R&D wing keeping in view the elements of quality by design which were -

Target Product Profile (TPP): defined as a "Prospective and dynamic summary of the quality characteristics of a drug product that ideally will be achieved to ensure that the desired quality, and thus the safety and efficacy, of a drug product are realized" and Target Product Quality Profile (TPQP)-a natural extension of TPP for product quality in order to reproducibly deliver the therapeutic benefit.

Critical Quality Attributes (CQA) - defined as "a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distributed to ensure the

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Table 1: Parameters

Sr. No.	Parameters	Data	RSD
1	Peak area	914160	0.92
2	Retention Time (min)	9.91	0.90
3	Theoretical plates	9931	0.99
4	Tailing factor	0.985	1.08

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

Parameters	**Glycyrrhizin**
Concentration range for linearity [µg ml^{-1}]	9.0 – 45
Correlation Coefficient (r2)	0.997
Amount of marker compound in Pulmofarm T [%] (w/w)[a]	0.36
Intermediate precision (Reproducibility) - RSD [%]Intraday 1	0.86
Interday 3	0.93
LOD	0.24 µg ml^{-1}
LOQ	0.71 µg ml^{-1}

[a] mean of 6 replicates

Table 3: Results from determination of recovery.

Parameter	Glycyrrhizin		
Initial concentration in formulation [mg g^{-1}]	3.6	3.6	3.6
Concentration added [mg g^{-1}]	0	2.0	4.0
Total concentration [mg g^{-1}]	3.6	5.6	7.6
Concentration found [mg g^{-1}]	3.55	5.52	7.68
RSD [%] (n=7)	0.90	0.96	0.98
Recovery [%]	98.61	98.57	101.05
Mean recovery [%]		99.41	

desired product quality" were identified and finished product specification was set to ensure the quality of product.

Process and product design and development cannot be separated since formulation cannot become a product without a process. Process design is the initial stage of process development where an outline of commercial manufacturing processes is identified including the intended scale of manufacturing. This includes all the factors that need to be considered for the design of the process, including facility, equipment, and material transfer and manufacturing variables.

The validation of manufacturing process of Pulmoform T required analysis of data gathered throughout the design and manufacturing in order to confirm that the process can reliably output product of a determined standard.

Out of four validation processes namely prospective process validation, concurrent process validation, retrospective process validation and revalidation we opted for concurrent process validation for our product's manufacturing. This validation involved in process monitoring of critical processing step i.e blending and helped us to generate and document evidence to show that the production process was in a state of control and the reproducibility of the production process will mainly ensure the batch-to-batch consistency of quality, efficacy and safety.

As a part of manufacturing process validation pre requisites, the protocol was designed with the objective to validate the manufacturing process of the product under study. The protocol specified how the process validation will be conducted, identifying critical steps & parameters to be monitored, sampling plan and acceptance criteria. As a part of protocol all the raw materials and packaging materials used in the manufacturing were procured from approved vendors. Testing was done and materials were accepted as per compliance to the respective specifications. A team comprising of F&D, Production, Engineering, ARD–R&D and QA was set with well assigned responsibilities to carry out all the activities as per protocol.

A flow chart showing al the manufacturing activities was prepared and shared with the team. Relevant SOPs (Standard Operating Procedure) were prepared and training was given to the relevant persons on equipment operation, manufacturing and sampling strategy.

The manufacturing equipment and control instruments used for manufacturing and analysis of the product were maintained as per GMP. All the instruments used in the process were duly calibrated as per the calibration schedule. The environmental conditions were considered as per pre defined acceptance criteria prior to conducting the process validation study.

A well designed sampling plan defining all the locations with time intervals from where the samples were to be collected was prepared and sampling was done accordingly. In total 81 samples were collected from the different positions of ribbon blender at the time interval of 15, 30 & 45 minutes.

Analytical method for the estimation of active content in the samples was developed as the integral part of the exercise at R&D. The method was validated on the basis of its selectivity, linearity, precision, accuracy, limit of detection and limit of quantification according to International Conference on Harmonization (ICH) guidelines.

Estimation of % active content Glycyrrhizin was carried out as per its validated analytical method. The process was supposed to be validated if % CV (Coefficient of Variance) is observed to be NMT 5 between the two extremes of % active content obtained after analysis.

MATERIAL AND METHODS

Reagents and materials

All the reagents and solvents were of AR or HPLC grade as per requirement. The active compound Glycyrrhizin

Table 4: Glycyrrhizin content in Pulmafarm

Sr. No.	Time of sampling	Repetitions	% w/w of Glycyrrhizin content in Pulmofarm –T samples								
			TR	TC	TL	MR	MC	ML	BR	BC	BL
1	15 min	A	0.41	0.40	0.39	0.42	0.40	0.42	0.39	0.39	0.38
		B	0.37	0.36	0.39	0.42	0.37	0.41	0.42	0.39	0.39
		C	0.39	0.39	0.42	0.41	0.42	0.38	0.39	0.42	0.37
		Mean	0.39	0.38	0.40	0.41	0.39	0.40	0.40	0.40	0.38
		CV	0.025								
		%CV	2.5								
2	30 min	A	0.41	0.40	0.39	0.41	0.38	0.39	0.38	0.41	0.38
		B	0.34	0.36	0.35	0.36	0.40	0.39	0.41	0.39	0.39
		C	0.39	0.37	0.38	0.35	0.42	0.39	0.34	0.39	0.32
		Mean	0.38	0.38	0.37	0.37	0.40	0.39	0.38	0.40	0.36
		CV	0.036								
		%CV	3.6								
3	45 min	A	0.34	0.39	0.35	0.42	0.41	0.41	0.42	0.39	0.38
		B	0.38	0.41	0.35	0.46	0.40	0.42	0.40	0.35	0.37
		C	0.41	0.41	0.44	0.4	0.42	0.35	0.34	0.43	0.43
		Mean	0.38	0.40	0.38	0.43	0.41	0.39	0.39	0.39	0.39
		CV	0.04								
		%CV	4.0								

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.

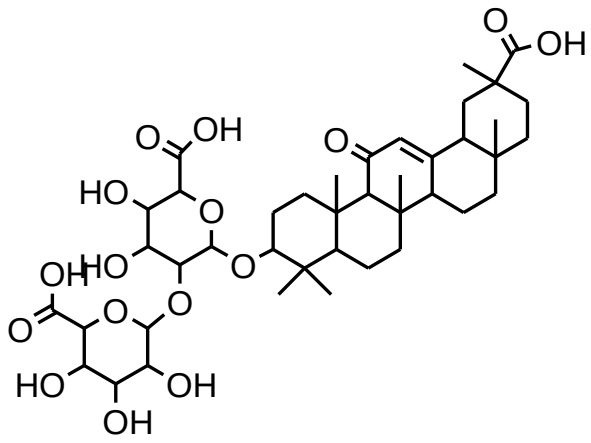

Figure **1**: Glycyrrhizin

was isolated in our lab and structure was established by interpreting the 1H, 13C & 2D NMR spectra, latest controlled samples of Pulmofarm-T were obtained from the QA/QC department of AYURVET LTD, Baddi.

Preparation of standard solution of Glycyrrhizin

Accurately weighed around 5 mg of standard Glycyrrhizin was dissolved in 5 ml of methanol to obtain stock concentrations of 1000 µg/ml. Stock solution was further diluted to obtain the dilution range of 9–45 µg/ml and then injected in HPLC in order to prepare the calibration graphs and quantification of bioactive.

Preparation of test solution

For the quantification of Glycyrrhizin, Pulmofarm-T (1g) was refluxed with 50 ml of petroleum ether (60ºC – 80ºC) for 3 hours and filtered, repeated the process one more time. The defatted sample was extracted with 50 ml of methanol under reflux conditions for 3 hours and filtered, repeated the process twice. The final volume was made to 100 ml with methanol, filtered the solution through 0.45 µm membrane filter before injecting into HPLC.

High Performance Liquid Chromatography

Apparatus and Conditions

Glycyrrhizin content was analyzed by High Performance Liquid Chromatography (WATERS, binary pump 515 with PDA 2996 detector, USA). The data was acquired on the Empower 2.0 controlling software. Separation was obtained on Phenomenex Luna C18 column (250 mm x 4.6 mm, 5µm).

Selection and Optimization of chromatographic condition

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for Glycyrrhizin (Fig. 1) was obtained by using Potassium dihydrogen phosphate buffer (5.3mM): Acetonitrile in 5:35, v/v ratio, pH 3.5 as a mobile phase in isocratic mode. The mobile phase was filtered through 0.45 µm Millipore filter and degassed before use. The flow rate was adjusted to 1.0 ml/min. Injection volume was adjusted to 20 µl and detection was made at 254 nm.

System suitability

The analytical results obtained by the method developed are only valid if the defined system suitability criteria are fulfilled. In this investigation, the experimental result (Table 1) indicates that the chromatographic system was suitable for intended analysis. Standard solution mixture

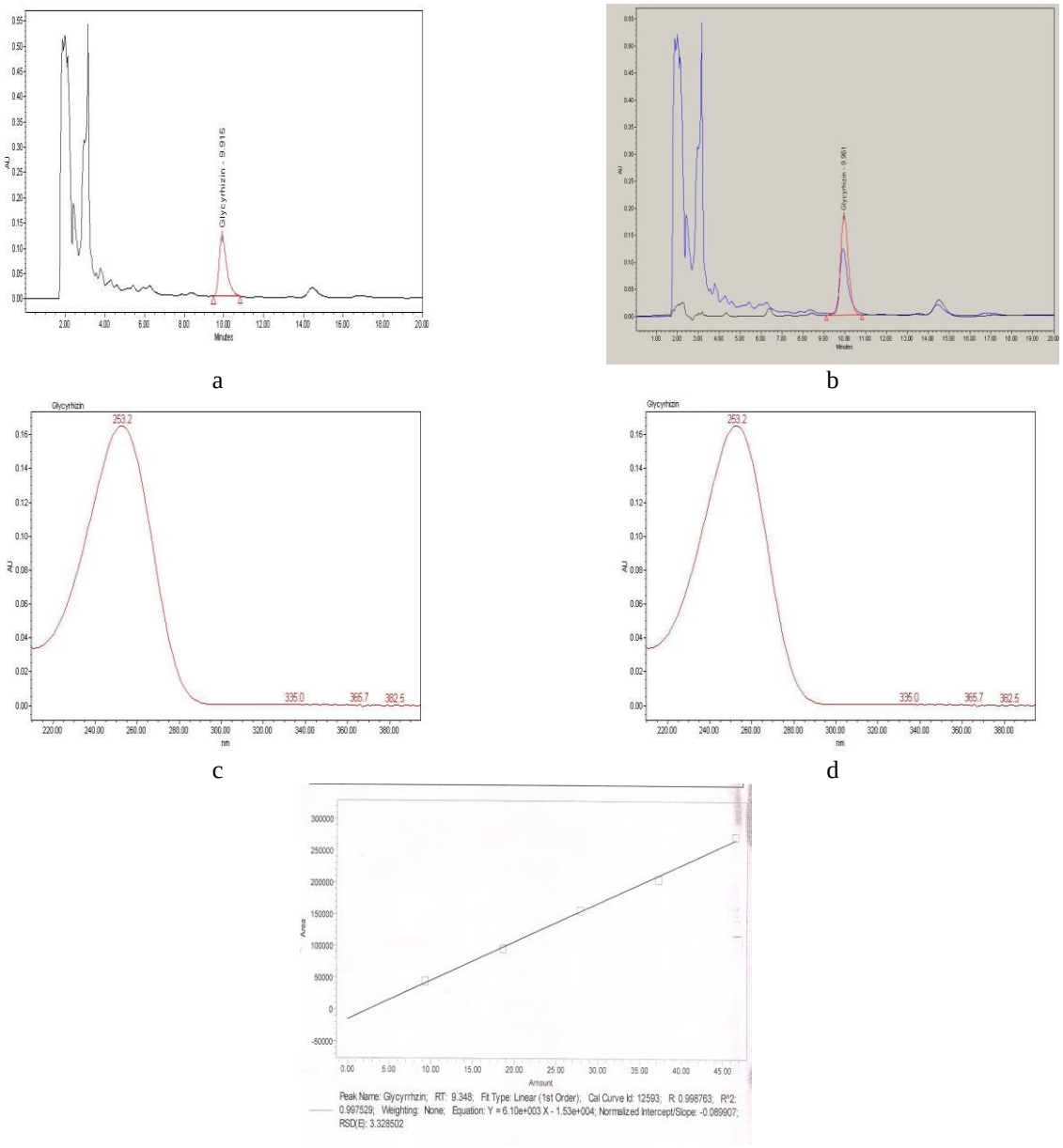

Figure 2: Chromatograms showing the resolution of marker compound in the formulation Pulmofarm -T. (a) Chromatogram of sample Pulmofarm-T. (b) Overlay of the Glycyrrhizin chromatograms i.e. sample against standard. (c) Spectral scan of standard Glycyrrhizin. (d) Spectral scan of Glycyrrhizin in Pulmofarm –T. (e) Calibration plot for Glycyrrhizin standard.

containing known concentration of Glycyrrhizin was injected six times, separately.

RSD values for peak area and retention time of standard suggested the reproducibility for these parameters. The low RSD values (Table 1) for tailing factor and theoretical plates suggested good peak symmetry of Glycyrrhizin and good efficiency of column.

Validation of the Method

The proposed method was validated for the determination of Glycyrrhizin using following parameters as per ICH guidelines:

Calibration: The marker compounds in the formulation were quantified using a calibration curve established with five dilutions of the standard. The corresponding peak area in formulation was plotted against the concentrations of the standard injected. Peak identification was achieved by comparison of both the retention time (RT) and UV absorption spectrum with those obtained for standard.

Linearity: Linear regression analysis was used to calculate the slope, intercept, and /regression coefficient (r2) for calibration plot. Linearity was determined by using five concentrations of the standard solution. Response was found to be linear in the concentration ranges investigated (Fig. 2: e, Table 2).

Range: Range is the interval between upper and lower concentration of analyte in sample for which it has been demonstrated that the analytical method has suitable level of precision, accuracy and linearity. The linear response

was observed over a range of 9-45 ppm (Fig. 2: e, Table 2).

Precision: Three different concentrations of marker compound solution in triplicates were
injected on three different times within the same day and repeating the same on three
different days to record intra-day and inter-day variations in the results. The low % RSD values of intraday and interday (Table 2) for the marker compounds Glycyrrhizin reveals that the proposed method is precise.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

For determination of limits of detection and quantification, different dilutions of the marker was injected with mobile phase as blank and determined on the basis of signal to noise ratio 3:1 and 10:1 respectively. The LOD and LOQ for the standard compounds were calculated and tabulated (Table 2).

Selectivity: The retention time of Glycyrrhizin and their counterpart in the formulation was 9.91 ±0.02 minute. The UV-Vis spectrum of marker compound was compared with its counterpart in formulation at three different positions, the peak start, peak center, and peak end. There was good correlation between spectra obtained at each of the three positions. The Glycyrrhizin peak was, therefore, not masked by any peak of other compound present in the formulation (Figures 2: c, d), which was indicative of peak purity.

Accuracy: Recovery experiments were conducted to check for the presence of positive or negative interferences from other ingredients/excipients present in the formulation and to study the accuracy of the method. Recovery was determined by the standard addition method. Glycyrrhizin standard was added to the formulation at two different concentrations, extraction and analysis was performed as described above. Recovery was calculated for each standard at each concentration (Table 3). The low value of relative standard deviation indicates that the proposed method is accurate.

RESULT AND DISCUSSION

Manufacturing process of Pulmofarm T Premix was taken up for the validation of blending time to ensure the consistency of product quality and justify the optimal time required to achieve it. Samples were collected as per the sampling plan, analyzed for Glycyrrhizin using RP-HPLC and found to be in the range of 0.32 % - 0.42 % (Table 4) . The manufacturing process of product gave a % CV i.e. percent coefficient of variance ranging from 2.5 – 4.0 at the 15, 30 & 45 minutes blending time intervals. The % CV=2.5 is achieved well within the first 15 minutes of blending and gets the rating of fair blending by standard norms and procedure applicable to blending of any particular formulation which mentions the % CV = 5.0 as the upper limit.

Quality Risk Assessment - Failure mode effect analysis (FMEA) approach as per ICH Q9 Quality Risk Management guideline was used to identify all potential variables. Raw material specifications of each individual herb was in place to control the quality of herb in the initial stage itself which otherwise could have an impact on a particular CQA.

Control Strategy – It ensures process performance and product quality through planned set of controls. Control of raw material attributes (e.g., herb raw material, excipients and primary packaging materials), FPS (finished product specifications), Procedural controls & Facility controls such as utilities, environmental systems and operating conditions were all taken care of to ensure the process validation.

Life cycle Management and Continuous improvement – CQAs shall be monitored on regular basis to ensure that the process is performing within the defined acceptable variability. As manufacturing experience of the product under consideration grows and opportunities for process improvement are identified, the operating space could be revised within the design space.

CONCLUSION

The manufacturing process stands validated as it met acceptance criteria and 15 minutes was concluded to be optimal blending time for uniformity of products active ingredients.

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