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RESEARCH ARTICLE

QUALITY ASSURANCE BY EFFECTIVE MANUFACTURING PROCESS VALIDATION OF BOVITUSS LIQUID A POLYHERBAL FORMULATION

Kotagiri Ravikanth, Anirudh Sharma and *Deepak Thakur

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

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ABSTRACT

Formulations must be manufactured to the highest quality levels. In the pharmaceutical industry, process validation performs this task, ensuring that the process does what it purports to do. It is an essential regulatory requirement. According to Food and Drug Administration (FDA), the goal of validation is "To Establish documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specification and quality attributes." Process validation is to create robust manufacturing process that consistently produces a drug product with minimum variation that adheres to quality criteria of purity, identity and potency. In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing as well as in developed countries owing to its natural origin and lesser side effects. Herbal medicines are being manufactured on the large scale in Pharmaceutical units, where manufacturers come across many problems such as availability of good quality raw material, authentication of raw material, availability of standards, proper standardization methodology of single drugs and formulation, quality control parameters. Therefore, at present quality assurance is one of the thrust area for the evaluation of traditionally vv used medicinal plants and herbal formulations. Phytochemical constituents present in the polyherbal formulation act as the critical quality attributes and control variables which are essential to carry out the process validation. 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. Thus control of the process from the beginning to the end and quality assurance along the complete process chain is the key which will ensure the batch to batch consistency of finished polyherbal products. Results proved that manufacturing process stands validated as it met acceptance criteria.

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INTRODUCTION

Facilities and processes involved in pharmaceutical production impact significantly on the quality of the products. The processes include raw material and equipment inspections as well as in-process controls. Process controls are mandatory in good manufacturing practice (cGMP). The purpose is to monitor the on-line and off-line performance of the manufacturing process, and hence, validate it. Thus validation is an integral part of quality assurance (Govindaraghavan, 2008 and Bent, 2008). General principles and approaches that FDA considers appropriate elements of process validation for the manufacture of human and animal drug and biological products, including active pharmaceutical ingredients (APIs or drug substances), collectively referred to in guidance as drugs or products. It was concluded from the study that the herbal medicinal industry is independently evolving its own rigid quality assurance and control systems using FDA and Good Manufacturing Practice (cGMP) guidelines ('Guidelines for the appropriate use of herbal medicines', 1998). This 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 System*. FDA has the authority and responsibility to inspect and evaluate process validation performed by manufacturers. The cGMP regulations for validating pharmaceutical (drug) manufacturing require that drug products be produced with a high degree of assurance of meeting all the attributes they are intended to possess. Effective process validation contributes significantly to assuring drug quality. The basic principle of quality assurance is that a drug should be produced that is fit for its intended use. This principle incorporates the understanding that the following conditions exist:

***Corresponding author*: Deepak Thakur,**
R&D Centre, Ayurvet limited, Village Katha, P.O. Baddi – 173205, District Solan, Himachal Pradesh, India

- Quality, safety, and efficacy are designed or built into the product.
- Each step of a manufacturing process is controlled to assure that the finished product meets all quality attributes including specifications.

In addition, the cGMP regulations require that facilities in which drugs are manufactured be of suitable size, construction, and location to facilitate proper operations. Equipment must be of appropriate design, adequate size, and suitably located to facilitate operations for its intended use. Automated, mechanical, and electronic equipment must be calibrated, inspected, or checked according to a written program designed to assure proper performance (ICH harmonized tripartite guideline, 2009 and 'Guidance for Industry Process Validation: General Principles and Practices', 2011). Many products are single-source or involve complicated manufacturing processes. Homogeneity within a batch and consistency between batches are goals of process validation activities. Validation offers assurance that a process is reasonably protected against sources of variability that could affect production output, cause supply problems, and negatively affect public health. It is suggested that the comprehensive specification for the herbal substance must be followed by a description and validation of the manufacturing process for the herbal preparation (Shinde, 2009). 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 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 (Groot, 2006). Bovituss Liquid is a proprietary polyherbal formulation of AYURVET LIMITED for livestock animals. It is clinically proven that Bovituss Liquid act as a supportive treatment for alleviating the clinical signs of respiratory distress and livestock animals suffering from respiratory infections. Bovituss Liquid is a scientific blend of various herbs and the clinical action produced by this is due to the varied action of the constituent herbs which act synergistically for better and faster recovery in respiratory tract infection. The key herbs present are *Adhatoda vasica* having antitussive (Jahan, 2007) and anti-bacterial (Karthikeyan, 2014) properties; *Ocimun sanctum* having immunomodulatory and anti-inflammatory (Mukherjee, 2005), properties; *Hedychium spicatum* showing bronchodilator, anti-inflammatory and analgesic activity (Ghildiyal, 2012); and *Glycyrrhiza glabra* showing anti-tussive, anti-inflammatory (Mirmala, 2011), and anti- bacterial (Nitalikar, 2010) activity. Various studies carried out have proved that the plant *Glycyrrhiza glabra* and it's one of the bioactive compounds Glycyrrhizin exhibits significant biological activities, hence the inclusion of the plant in formulation will add value to its bio efficacy. 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. A flow chart showing all the manufacturing activities was prepared and shared with the team. Standard operating procedure (SOPs) 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 cGMP. 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 18 samples were collected from the different positions of mixing tank at the time interval of 10, 15 & 20 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 ('Guidance for Industry Process Validation: General Principles and Practices', 2011). Estimation of % active content Glycyrrhizin was carried out as per its validated analytical method. The process was supposed to be validated if percentage coefficient of variance (CV) is observed to be NMT 5 between the two extremes of percentage active content obtained after analysis.

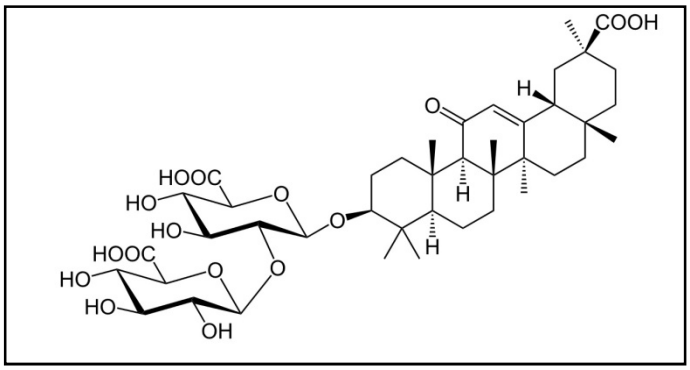

Fig. 1. Glycyrrhizin

MATERIAL AND METHODS

Reagents and materials: All the reagents and solvents were of AR or HPLC grade as per requirement. The active reference compound Glycyrrhizin was procured from the Sigma aldrich, latest controlled samples of Bovituss Liquid 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 50 ml of methanol to obtain stock concentrations of 100 µg/ml. Stock solutions was further diluted to obtain the dilution range of 10–80 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, Bovituss Liquid (10g) was sonicated with 35 ml of HPLC grade methanol for 20 minutes and filtered. The final volume was made to 50 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 (KH_2PO_4) 5.3 mM: Acetonitrile in 65:35, v/v ratio, pH 3.5 with acetic acid 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.

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.

Table 1. Chromatographic parameter

Sr. No	Parameter	Data	RSD
1	Peak Area	1344975	0.10
2	Retention Time (min)	24.70	0.90
3	Theoretical Plates	12541	0.97
4	Tailing factor	0.983	0.97

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

Sr. No.	Parameters	Glycyrrhizin
+1	Concentration range for linearity [µg ml-1]	10.0 – 80.0
2	Correlation coefficient (r2)	0.997
3	Amount of marker compound in Bovituss Liquid [%] (w/w)	0.030
4	Intermediate precision (Reproducibility)-RSD [%] Intraday	0.24
5	Interday	0.27
6	LOD [µg ml-1]	0.24
7	LOQ [µg ml-1]	0.71

Table 3. Results from recovery analysis

Sr. no.	Parameter	Glycyrrhizin		
1	Initial concentration in formulation [mg g-1]	0.30	0.30	0.30
2	Concentration added [mg g-1]	0	2.0	4.0
3	Total concentration [mg g-1]	0.30	2.30	4.30
4	Concentration found [mg g-1]	0.29	2.18	4.22
5	RSD [%] (n=7)	0.92	0.98	0.97
6	Recovery [%]	96.66	94.78	98.14
7	Mean recovery [%]	96.53		

Table 4. Glycyrrhizin content in Bovituss Liquid

Sr. No.	Time of sampling	Repetitions	% w/w of Glycyrrhizin content in Bovituss Liquid Batch No 18001					
			U1	U2	U3	L1	L2	L3
1	10 min		0.023	0.021	0.021	0.021	0.027	0.026
		Mean	0.022			0.025		
		CV	0.0903					
		% CV	0.903					
2	15 min		U1	U2	U3	L1	L2	L3
			0.038	0.036	0.037	0.034	0.034	0.033
		Mean	0.037			0.034		
		CV	0.0598					
		% CV	0.598					
3	20 min		U1	U2	U3	L1	L2	L3
			0.029	0.029	0.027	0. 034	0.034	0.033
		Mean	0.028			0.034		
		CV	0.1369					
		% CV	1.3					

U1- upper 1, U2- upper 2 & U3-Upper 3
L1- Lower 1, L2-Lower2 & L3-Lower3

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 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.

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: d, 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 10-80 ppm (Fig. 2: d, Table 2).

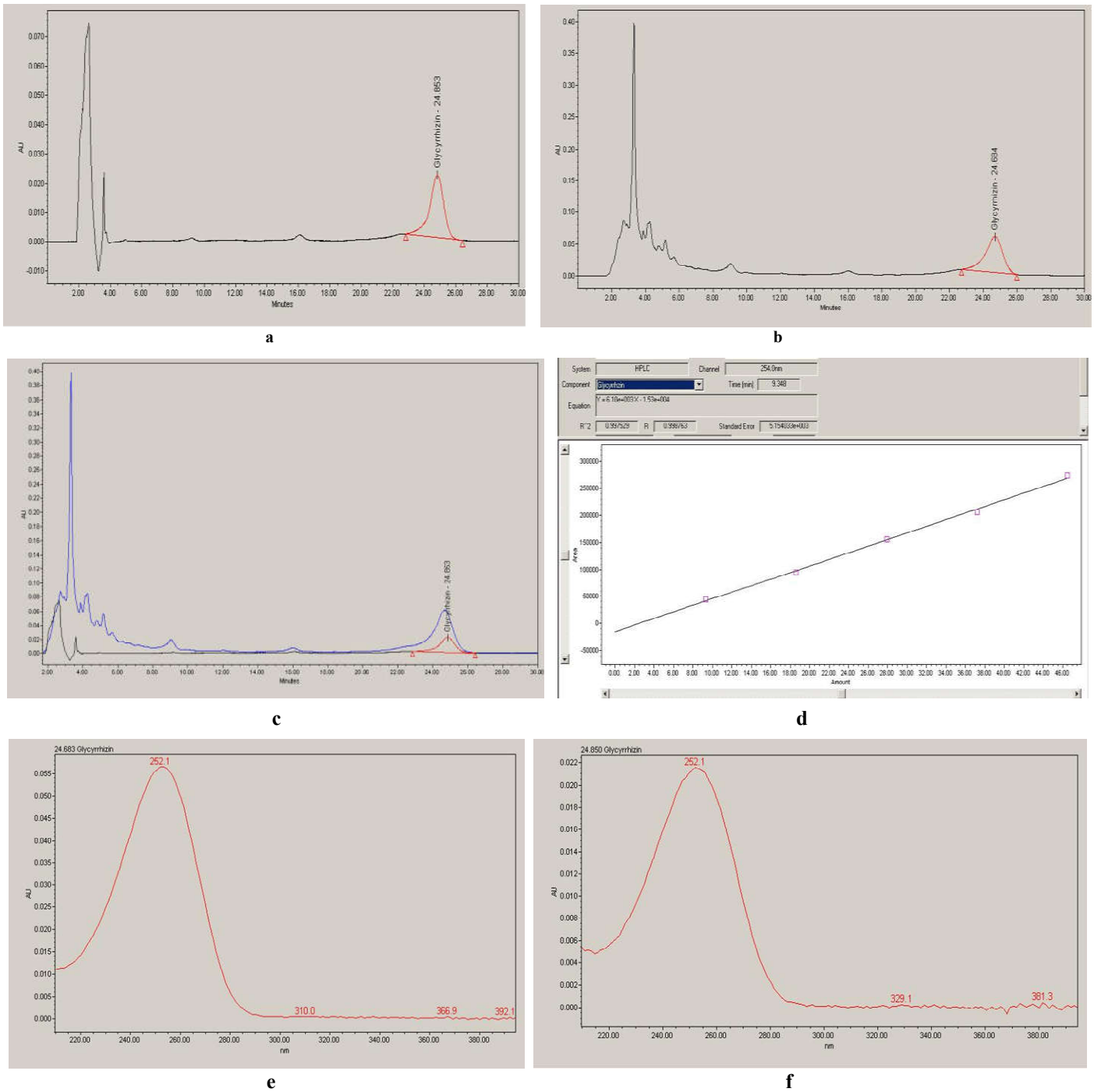

Fig. 2. Chromatograms showing the resolution of marker compound in the formulation. (a) Chromatogram of standard Glycyrrhizin. (b) Chromatogram of sample Bovituss Liquid. (c) Overlay of the Glycyrrhizin chromatograms i.e. sample against standard. (d) Calibration plot for Glycyrrhizin standard. (e) Spectral scan of standard Glycyrrhizin. (f) Spectral scan of Glycyrrhizin in Bovituss Liquid

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 24.80 ±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: e,f), 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.

RESULTS AND DISCUSSION

Manufacturing process of Bovituss Liquid was taken up for the validation of mixing time to ensure the consistency of product quality and justification of 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 0.021% - 0.038% (Table 4). The manufacturing process of product gave a % CV i.e. percent coefficient of variance ranging from 0.903 – 1.3 at the 10, 15 & 20 minutes mixing time intervals. The % CV=0.598 is achieved well within the first 15 minutes of mixing and gets the rating of fair mixing by standard norms and procedure applicable to mixing 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 critical quality attributes.

Control Strategy: It ensures process performance and product quality through planned set of controls. Control of raw material (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: Critical quality parameters 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 mixing time for uniformity of products active ingredients.

Declarations
Funding: None.
Conflict of interest: None declared
Ethical approval: Not required

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