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QUALITY ASSERTIVENESS BY PROSPECTIVE PROCESS VALIDATION OF TOXIROAK GOLD PREMIX A POLYHERBAL FORMULATION

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ABSTRACT

Validation study first proposed by Food and Drug Administration (FDA) and is a concept that is fundamental to Good Manufacturing Practice (GMP) and any quality assurance program. Validation of the individual steps of the process is called process validation. It is necessary for the efficient use of the resources. The study incontrovertibly leads to an objective of process optimization, better productivity and lower manufacturing cost. Validation concept is somewhat less implemented in herbal industries. It is necessary to development of modern and objective standards for evaluating quality of herbal medicines. So there is an urgent need of process validation in manufacturing of herbal drugs to improvise the quality of herbal drugs. Phytochemical constituents present in the polyherbal formulation act as critical quality attributes and control variables which are essential to carry out the process validation. 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. 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.

KEYWORDS: Process Validation, HPLC, Rutin, Toxiroak Gold premix.

INTRODUCTION

Validation is defined as the collection and evaluation of data, from the process design stage through commercial production, which establishes scientific evidence that a process is capable of consistently delivering quality product.

USFDA defined validation as "establishing documented evidence which provides high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality characteristics." Assurance of product quality is derived from careful attention on number of factors including selection of quality materials, adequate product and process design, control of process and in process and end product testing.

Validation concept is applied in pharmaceutical industry, but not that much deeply methodologically studied in herbal industry. All international regulations like USFDA, MCC, MHRA, TGA etc. shows the applicability of validation to pharmaceutical manufacturing but no one regulation except WHO applies the validation concept to manufacturing of herbal drugs. Therefore introduction of scientific validation would control the production of impure and adulterated herbal product and will ensure the batch to batch consistency of quality.[1]

Process validation is to create a robust manufacturing process that consistently produces a drug product with minimum variation that adheres to quality criteria of purity, identity and potency. It is generally carried out on 3 to 4 consecutive batches of product. In process test and finished product tests are conducted and it gives assurance of quality of final product.

To carry out process validation, it is essential to determine critical quality attributes and control variables. The process is validated based on these parameters. ICH Q8 guidelines of pharmaceutical development describes about role of critical quality attributes in process validation. 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. CQAs are generally associated with the drug substance, excipients, intermediates and drug product.[2] Potential drug product CQAs derived from the quality target product profile and/or prior knowledge is used to guide the product and process development. 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. Relevant CQAs can be identified by an iterative process of quality risk management and experimentation that assesses the extent to which their variation can have an impact on the quality of the drug product.[3]

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.[4]

Toxiroak Gold premix, a proprietary polyherbal formulation of AYURVET for poultry, is a mycotoxins inhibitor and acts as a mould inhibitor; it inhibits the biosynthesis of mycotoxins, effectively binds the toxins in the GI tract and helps in bioneutralization of the toxins in liver. The mould inhibition and bio-neutralization activity of Toxiroak is due to the presence of certain herbs like *Azadirachta indica, Curcuma longa, Allium sativum* that are known to inhibit the growth of fungus and block the biosynthesis of mycotoxins.

Various studies carried out have proved that the plant *Azadirachta indica* and it's one of the bioactive compounds Rutin exhibits significant biological activities, hence the inclusion of the plant in formulation will add value to its bio efficacy.

Manufacturing process of Toxiroak Gold Premix was undertaken for its validation as part of quality by design (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 desired product quality" were identified and finished product specification was set to ensure the quality of product.

The validation of manufacturing process of Toxiroak Gold Premix 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. 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 of quality and its reproducibility.

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 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.[5]

Estimation of % active content Rutin 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.

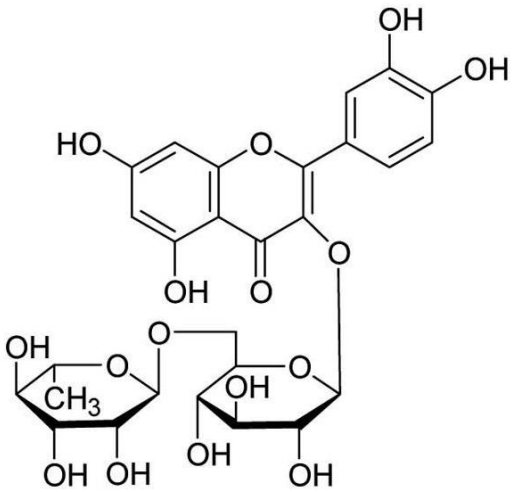


Fig 1: Rutin

MATERIAL AND METHODS

Reagents and materials

All the reagents and solvents were of AR or HPLC grade as per requirement. The active reference compound Rutin was procured from the Sigma aldrich, latest controlled samples of Toxiroak Gold premix were obtained from the QA/QC department of AYURVET LTD, Baddi.

Preparation of standard solution of Rutin

Accurately weighed around 5 mg of standard Rutin was dissolved in 50 ml of methanol to obtain stock concentrations of 100 µg/ml. Stock solution 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 Rutin, Toxiroak Gold Premix (5g) 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

Rutin 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 Rutin (Fig. 1) was obtained by using Water: Methanol in 60:40, v/v ratio, pH 2.8 with Ortho phosphoric 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 257 nm.

Table 1: Chromatographic parameter.

Sr. No	Parameter	Data	RSD
1	Peak Area	1879901	0.10
2	Retention Time (min)	16.56	0.90
3	Theoretical Plates	12541	0.96
4	Tailing factor	0.981	0.98

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

Sr. No.	Parameters	Rutin
1	Concentration range for linearity [µg ml^{-1}]	10.0 – 80.0
2	Correlation coefficient (r2)	0.999
3	Amount of marker compound in Toxiroak Gold Premix [%] (w/w)[a]	0.031
4	Intermediate precision (Reproducibility)-RSD [%] Intraday 1	0.94
5	Interday 3	0.93
6	LOD [µg ml^{-1}]	0.0037
7	LOQ [µg ml^{-1}]	0.011

[a]*mean of 6 replicates.*

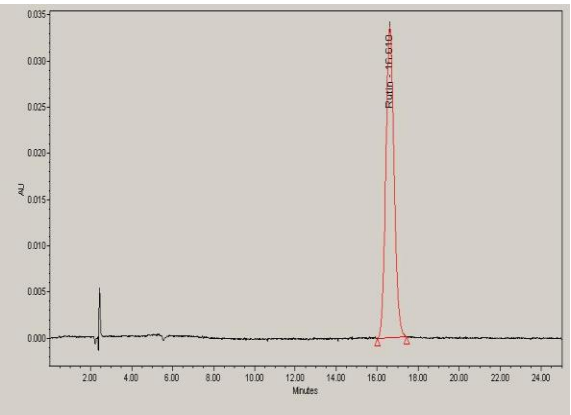
Table 3: Results from recovery analysis.

Sr. No.	Parameter	Rutin		
1	Initial concentration in formulation [mg g^{-1}]	0.31	0.31	0.31
2	Concentration added [mg g^{-1}]	0	2.0	4.0
3	Total concentration [mg g^{-1}]	0.31	2.31	4.31
4	Concentration found [mg g^{-1}]	0.30	2.18	4.17
5	RSD [%] (n=7)	0.91	0.96	0.97
6	Recovery [%]	96.77	94.37	96.75
7	Mean recovery [%]	95.96		

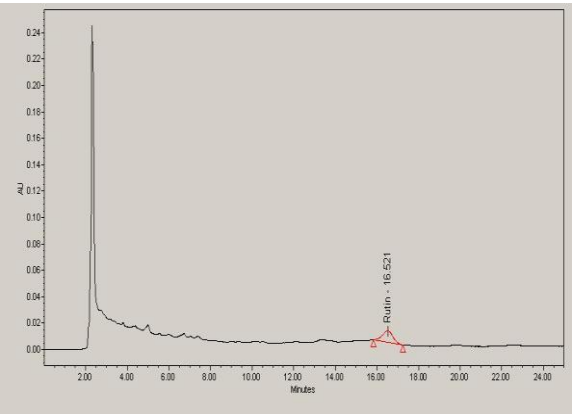
Table 4: Rutin content in Toxiroak Gold Premix

Sr. No.	Time of sampling	Repetitions	% w/w of Rutin content in Toxiroak gold powder								
			TR	TC	TL	MR	MC	ML	BR	BC	BL
1	15 min	A	0.041	0.025	0.028	0.022	0.023	0.022	0.041	0.023	0.022
		B	0.040	0.031	0.024	0.022	0.027	0.023	0.040	0.025	0.022
		C	0.033	0.029	0.027	0.025	0.026	0.023	0.033	0.025	0.025
		Mean	0.038	0.028	0.026	0.023	0.025	0.023	0.038	0.024	0.023
		CV	0.2231								
		% CV	**22.31**								
2	30 min	A	0.030	0.031	0.033	0.030	0.032	0.029	0.031	0.028	0.029
		B	0.030	0.031	0.026	0.030	0.029	0.040	0.031	0.035	0.032
		C	0.029	0.029	0.032	0.029	0.029	0.029	0.029	0.027	0.032
		Mean	0.030	0.030	0.030	0.030	0.030	0.033	0.030	0.030	0.031
		CV	0.0333								
		% CV	**3.33**								
3	45 min	A	0.030	0.030	0.026	0.030	0.027	0.027	0.027	0.028	0.035
		B	0.023	0.037	0.027	0.023	0.030	0.033	0.030	0.026	0.041
		C	0.032	0.027	0.027	0.032	0.024	0.030	0.024	0.026	0.026
		Mean	0.028	0.031	0.027	0.028	0.027	0.030	0.027	0.027	0.034
		CV	0.0847								
		% CV	**8.47**								

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.



(a)



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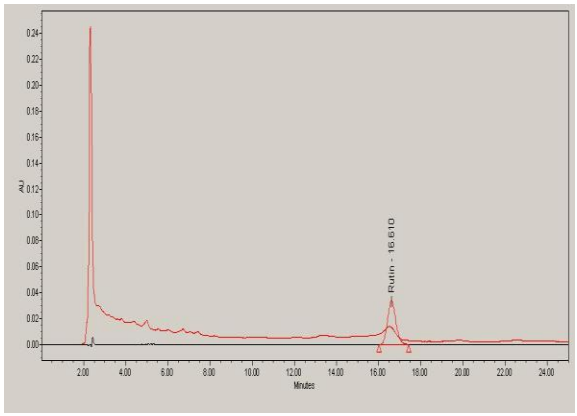

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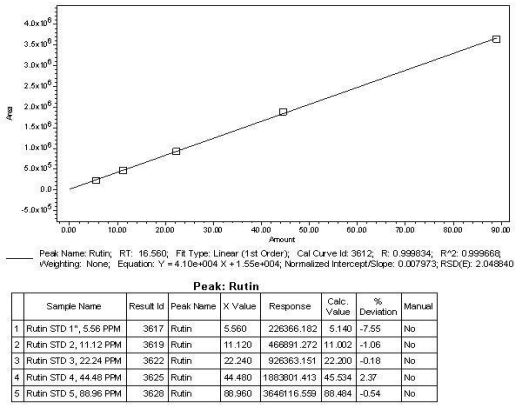
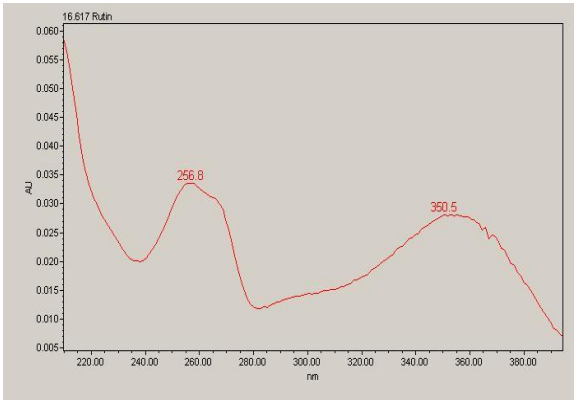


Peak: Rutin

	Sample Name	Result Id	Peak Name	X Value	Response	Calc. Value	% Deviation	Manual
1	Rutin STD 1*, 5.56 PPM	3617	Rutin	5.560	226366.182	5.140	-7.55	No
2	Rutin STD 2, 11.12 PPM	3619	Rutin	11.120	466891.272	11.002	-1.06	No
3	Rutin STD 3, 22.24 PPM	3622	Rutin	22.240	926363.151	22.200	-0.18	No
4	Rutin STD 4, 44.48 PPM	3625	Rutin	44.480	1883801.413	45.534	2.37	No
5	Rutin STD 5, 88.96 PPM	3628	Rutin	88.960	3646116.559	88.484	-0.54	No

(d)



(e)

(f)

Fig 2: Chromatograms showing the resolution of marker compound in the formulation. (a) Chromatogram of standard Rutin. (b) Chromatogram of sample Toxiroak Gold premix. (c) Overlay of the Rutin chromatograms i.e. sample against standard. (d) Calibration plot for Rutin standard. (e) Spectral scan of standard Rutin. (f) Spectral scan of Rutin in Toxiroak Gold Premix.

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 Rutin 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 Rutin and good efficiency of column.

Validation of the Method

The proposed method was validated for the determination of Rutin 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: 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).

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 Rutin 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 Rutin and their counterpart in the formulation was 16.60 ±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 Rutin 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. Rutin 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 Toxiroak Gold 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 Rutin using RP-HPLC and found to be in the range of 0.022% - 0.041% (Table 4). The manufacturing process of product gave a % CV i.e. percent coefficient of variance ranging from 3.33 – 22.31 at the 15, 30 & 45 minutes blending time intervals. The % CV=3.33 is achieved well within the first 30 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 30 minutes was concluded to be optimal blending time for uniformity of products active ingredients.

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REFERENCES

1. Desai S R, Disouza J I, Shirwadkar B B. "Process Validation: An Approach for Herbal Tablet Standardization", International Journal of Pharmacognosy and Phytochemical Research 2016; 8(2): 313-320.
2. Desai L, Oza J, Khatri K. "Prospective process validation of polyherbal cough syrup formulation", Journal of Advanced Pharmacy Education & Research, 2012; 2(4): 225 -231.
3. 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.
4. 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.
5. ICH harmonized tripartite guideline, Q8 Guidelines for Pharmaceutical Development, Revision 2, Geneva, Switzerland; 2009; 12-14.