IJESRT

INTERNATIONAL JOURNAL OF ENGINEERING SCIENCES & RESEARCH TECHNOLOGY

Optimisation And Method Validation of Invitro Determination of Adapalene In Adapalene 0.1% Gel Formulation By Manual Diffusion Cell.

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Abstract

One of the most critical factors in developing pharmaceutical drug substances and products is ensuring that the analytical test methods used to analyze pharmaceutical products should generate valid and meaningful data in terms of reliability, accuracy and precision, regardless of whether it is intended for acceptance, release, stability or pharmacokinetic studies. Validation of an analytical method is a process that provides documented evidence that an analytical test method performs in an appropriate manner for the purposes for which it was intended. Method validation is a process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistence of analytical results; it is an integral part of any good analytical practices. The USP has published the specific guidance for method validation for compound evaluation.USP defines the steps for validation as accuracy, precision, linearity, ruggedness, robustness.

Keywords:-Method validation, in vitro release (IVR), manual diffusion cell, synthetic support membranes, receptor medium, Reverse Phase-High Performance Liquid Chromatography (RP-HPLC).

Introduction

One type of network sees the nodes as ‘artificial A variety of general validation protocols have been recommended by many organizations such as the FDA1, United States Pharmacopeia (USP) 2 and the International Conference on Harmonization (ICH).3,4 The FDA requires a manufacturer of a pharmaceutical product to establish and document the accuracy, sensitivity, specificity and reproducibility of the analytical method.5 The USP specifies typical performance characteristics such as accuracy, precision, linearity, range and sample stability that should be considered in the validation of analytical methods intended for the analysis of finished pharmaceutical products. A review and a strategy for the validation of analytical methods for method developed should be produced during method validation. It is important that the main objective of validation of an analytical procedure is to demonstrate that the procedure is suitable for its intended purpose.

The important factors in developing pharmaceutical drug substances and products is ensuring that the analytical test methods used generates valid and meaningful data in terms of reliability, accuracy and precision, regardless of whether it is intended for acceptance, release, stability study. Validation of an analytical method is a process that provides evidence that an analytical test method performs in an appropriate manner for the desired purposes. By considering all the above facts, all the validation parameters were studied for diffusion method of adapalene gel (0.1%) formulation. The parameters which are being included in the validation study are discussed in results and discussion.

Linearity is an indication of the capability of a test method to produce test results that are directly proportional to the amount of analytes in a sample within a given concentration range.6The accuracy of a method was determined by analyzing a sample of known concentration and comparing the measured value to the true value or comparing test results from alternate well-characterized procedure. System precision or intra-day precision of an analytical method is an indication of the performance of an analytical procedure conducted within a laboratory over a short time interval using the same analyst with similar equipment. Analytical method precision has provided an indication of the variability of analytical results as a function of the analyst, manipulation of samples and the day-to-day environment in which the method was applied.7 An intermediate precision was performed on the different instruments and by

different analyst. Robustness parameter was studied for the critical variant such as variation in composition of diffusion medium. The method used for diffusion study is discussed as follows.

Results And Discussion

Diffusion method for validation *of in vitro* release of adapalene

The diffusion method was developed as per the in vitro release test condition that there should not be more than 30 % release of the cream in the diffusion medium. For diffusion study, the hydro alcoholic medium (65:35) v/v and tuffryn membrane was used as a synthetic support membrane. The diffusion is studied using manual diffusion cell maintained at 32 ± 2°C. Approximately 300 mg of cream containing 0.3 % of adapalene API was used for experiment. Samples were analysed by RP-HPLC method.[8]

The previously soaked membranes in isopropyl myristate was attached to the dosage wafer from the lower side. Approximately 300 mg of cream was filled in the central hole of the dosage wafer. The sample was spread uniformly on the dosage wafer with the help of Teflon spreader. This membrane along with sample is kept in contact with the receptor medium. The dosage wafer was covered with glass disk to prevent solvent losses due to evaporation from the cream. Membrane, dosage wafer and glass disk was kept in alignment with help of alignment ring. This ring was used to align membrane and glass disk and also used to fit these components compactly. All the above components such as alignment ring, glass disk and membrane were fitted using an aluminium clamp. All the remaining six manual diffusion cells were prepared in similar way. The internal standard solution of 40 mg ml^{-1} of adapalene API was prepared considering highest release of the cream in receptor medium. Samples were taken at an interval of 0.0, 1.0, 2.0, 4.0, 6.0, 8.0 hours and analysed along with internal standard using RP-HPLC method. The required quantity of sample for analysis was 2.0 ml. The sample was collected from sample port in HPLC vial by pushing the fresh medium through replace port using the calibrated syringe. Dyffryn gel from Galderma Pharmaceuticals USA was used as a reference formulation for the validation study. The purity of this formulation is also verified. For linearity and accuracy study adapalene API was used.

Chromatographic separation was performed on high performance liquid chromatography (Waters, Inc.) with 2695 separation module and 2487 dual λ absorbance detector system. Chromatographic separation was performed using Phenomenex, Luna RP-150 mm X 4.6 mm X 5µ 100-3 column and mobile phase comprising a mixture triflouroacetic acid buffer (0.1%) and acetonitrile (20:80) v/v, at a flow rate of 1.0 ml min-1 and UV detection at 225 nm. Samples were injected in volumes of 50µl. Peak purity of drug was checked using photo diode array detector. Chromatograms and data were recorded by using Empower software. For evaluation of validation parameters the Adapalene USP standard (99.98% w/w) was used as an internal standard.

The above method discussed is used to evaluate the various validation parameters. The results obtained during the validation of diffusion method are discussed as follows.

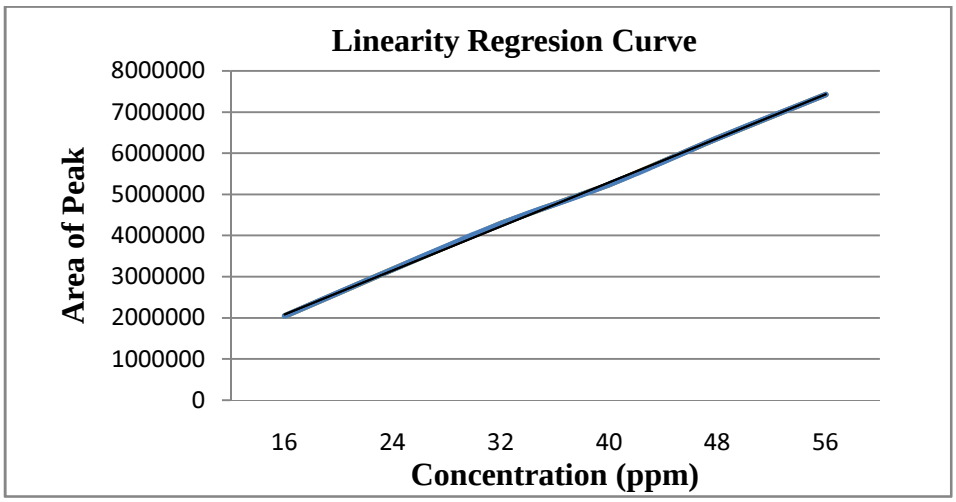
Linearity:-A linearity Calibration standards ranging from 40 to 140% (16, 24, 32, 40, 48, 56) ppm were prepared. The calibration curve in these studies was found to be linear with R^2 value = 0.999, a slope of 133638 and a y-intercept of -52782.819 yielding an equation for the calibration line of y = -52783 x– 133638.The linearity regression curve is shown in figure 1.Linearity was tested using least squares linear regression analysis of the peak area versus concentration data is show in the table 1.

Table 1 –Linearity regression factor against different concentration levels.

Sr. No.	Concentration Level (%)	Concentration(PPM)	Area of Peak	R2 Correlation
1	40	16	2043890	0.999
2	60	24	3180916	
3	80	32	4290116	
4	100	40	5242396	
5	120	48	6365398	
6	140	56	7426500	

Least squares linear regression analysis is shown in figure 1.

Figure 1:-Linearity regression curve for chromatographic method.

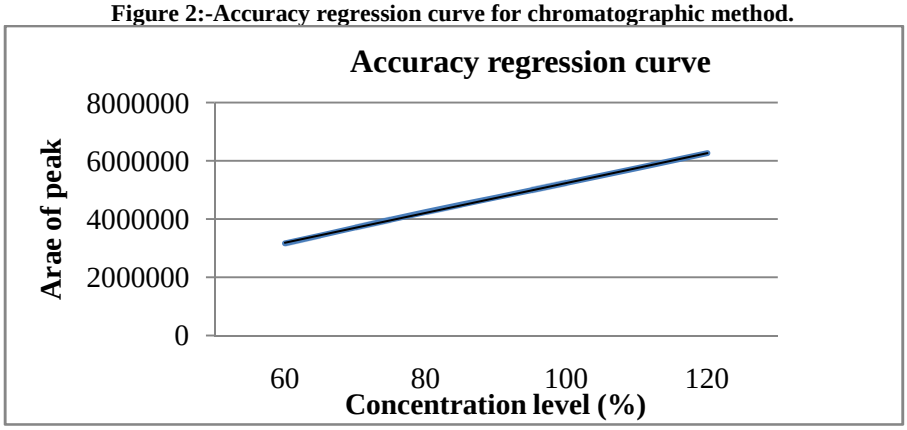


Accuracy: The known amounts of internal calibration standard spiked with known weight of the placebo i.e. 90 mg in mobile phase were prepared. Three samples representing low (24,32 ppm), medium (40ppm) and high (48ppm) concentrations were prepared and injected. The R^2 value of accuracy is found to be 0.999. The linearity regression curve is shown in figure 2.Accuracy was tested using least squares linear regression analysis of the peak area versus concentration data is show in the table 2.The accuracy correlation curve is shown in figure 2.

Table 2 – Percentage recovery and correlation factor for accuracy study.

Sr. No.	Concentration Level (%)	Conc of API (ppm)	% recovery	Corr. Factor (R2)
1	60	24	100.49	0.999
2	80	32	101.05	
3	100	40	99.86	
4	120	48	99.51	

Figure 2:-Accuracy regression curve for chromatographic method.



Precision:-System, method and intermediate Precision study of adapalene (0.1%) cream indicates the performance of diffusion method. Method precision study was carried out by different analyst, manipulation of samples and altering the laboratory conditions. For an intermediate precision, method was performed on the different instruments and by other analyst to eliminate the chances of environment factors and errors for two consecutive days. For system precision the diffusion study was performed on the six sample manual diffusion cell in order to study the repeatability. The intermediate precision study was also performed on all the sample manual diffusion cells but at different days and by different analyst. The repeatability of this method was assessed by calculating the % release of adapalene formulation. The results obtained during the precision study are shown in the Table 3.

Table 3:-Percentage recovery of internal standard for system precision.

Sr.no	Precision	% Release
1.	System precision	29.94
2.	Method Precision	30.33
3.	Intermediate precision	Day 1:- 29.47
		Day 2:- 30.02

Robustness:-The hydroalcoholic medium is very volatile at 32°C .There are chances of evaporation of ethanol from diffusion medium over the length of the experiment. Therefore, this may alters the composition of diffusion media and ultimately affects the *in vitro* release pattern of the cream. Therefore, this robustness parameter helps to observe the effect of change in the diffusion medium. The effect of change of diffusion medium along with experimental composition is shown in the table-4.

Table 4:-Percentage recovery of internal standard for robustness study.

Sr. No	diffusion Medium composition (hydro alcoholic medium)	% release of the Adapalene cream
1	(60:40) v/v	26.69
2	(70:30) v/v	24.56

Conclusion

The diffusion invitro release method was validated according to USP, FDA and ICH guidelines. The regression factor for linearity, accuracy and in vitro release in terms of µg/cm^2 was found to be suitable for precise, selectivity sensitivity; robustness. The method is suitably validated for the intended purpose of adapalene (0.1%) gel invitro release study. The linearity, precision, accuracy of the method proves that the method is specific, accurate, easily reproducible, robust and can be used for the determination of in vitro release of adapalene in adapalene 0.1 % formulations.

The linearity was carried out by using the active pharmaceutical ingredient of adapalene cream formulation. The linearity regression curve in figure1 shows a linear relationship of recovery of adapalene with increasing concentrations of active pharmaceutical ingredient. This indicates that the method can be used suitably upto 140 concentration level if only active pharmaceutical ingredient is under the study. The placebo in the formulation may interfere the linearity relationship of the method. In order to study these effects the accuracy study is carried out. The known concentration level of API is spiked with 90 mg of placebo and samples were prepared in mobile phase. The recovery of API in the placebo matrix is studied. The table 2 shows a percentage recovery of API in placebo matrix. By establishing the linearity and accuracy correlations, the evaluations of other validation parameters such as precision, robustness becomes easier for evaluation.

The validation of system precision shows the uniform recovery of in vitro release pattern of the cream as well as uniform performance of the other experimental parameters such as agitation speed, composition of diffusion medium and temperature. Intermediate precision data shows that the method has good repeatability when instrument, analyst are change and if experiment is carried out at different day. From the data of intermediate precision, it can be interpret that the method has good control over the different environment conditions, human errors and instrumental errors.

During the robustness study, it is observed that, since adapalene is less soluble in water, slight increase in the concentration of aqueous phase in the diffusion medium altered the results drastically. i.e. *in vitro* release was directly drops to 26.69 %.The composition of diffusion medium is very critical parameter for the cream to release in the diffusion medium Thus, the variation in the diffusion media is not a rugged parameter for the diffusion method. This indicates that the change in the composition of diffusion media will affect the diffusion pattern adversely. Thus, at any condition, the diffusion medium composition should not be altered. Thus, the validation study helps to evaluate the different variants and critical factors of the experiments which may alter the results. This method can be used as a quality control tool under the drastic experimental conditions.

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