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Research Article

Development and Validation Of RP- HPLC Method for the Estimation of Imiquimod in Pharmaceutical Dosage Forms

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ABSTRACT

A reverse phase liquid chromatography (RP-HPLC) method has been developed and subsequently validated for the determination of Imiquimod. Separation was achieved with a cosmosil C18, 250 mm × 4.6 mm I.D; particle size 5 µm) Column and pH 4.6 phosphate buffer and Acetonitrile (20:80) v/v as mobile phase at a flow rate of 0.8 mL/min and the Column temperature was maintained at 25°C. UV detection was performed at 244 nm with a run time of 10 min. The method is simple, rapid, and selective. The described method of imiquimod is linear over a range of 5 ng/mL to 600 ng/mL and with correlation coefficient of 0.992 respectively. The method precision for the determination of assay was below 2.0% RSD. The method enables accurate, precise, and rapid analysis of imiquimod.

Keywords: RP-HPLC, Imiquimod, UV, Column, Rapid analysis

INTRODUCTION

Now day's pharmaceutical industries are showing increasing interest in topical preparations i.e. creams, ointments, lotions, foams, gels and nasal sprays etc. For accurate analysis of any pharmaceutical dosage form, simple, rapid and reproducible analytical methods are required. Liquid chromatographic separation technique is a powerful analytical tool and most preferable analytical technique used in pharmaceutical industries [1-6]. The developed analytical method should be accurate, reproducible, robust, precise and commercially viable one [7]. To ensure all these parameters in a method, validation of the analytical method is required as per International Conference on Harmonization (ICH) guidelines [8,9]. Imiquimod cream is commonly used to treat genital warts, known as Human Papilloma Virus (HPV). It is also used as a treatment of precancerous skin lesions, known as actinic keratosis. It works by increasing the body's immune system to fight viral infections. The cream is effective for treating the warts or lesions without scarring the skin [10]. Chemical structure of Imiquimod is shown in (Figure 1)

Literature survey revealed that there is no any HPLC method reported for determination of imiquimod content in imiquimod cream. For imiquimod active pharmaceutical ingredient (API) and for some biological samples, few methods were reported but no method has been reported for imiquimod topical preparations (imiquimod creams). This proposed method is very simple and rapid for quality analysis of imiquimod content in imiquimod cream.

MATERIALS AND METHODS

Materials

Imiquimod is obtained as gift sample from Dr.Reddy's Lab, Hyderabad. Methanol, Phosphate buffer, Acetionitril were purchased from Sd Fine chemicals Limited. All other chemicals used in the study were analytical grade. Imiquimod standard and cream samples were obtained as a gift samples from sipra limited. Ortho phosphoric acid (GR grade), triethyl amine (GR Grade), potassium dihydrogen phosphate, hydrochloric acid (GR Grade), were purchased from qualigens. HPLC grade Acetonitrile was obtained from Rankem. Auto sampler high performance liquid Chromatograph Shimadzu 2010 equipped with software "class-vp" along with UV and PDA detector was used.

Chromatographic conditions

Mobile phase was a mixture of phosphate buffer pH 4.6 and Acetonitrile (20:80) v/v. Mobile phase was filtered through a 0.45 µm nylon filter and degassed for 5 min using an ultrasonicator. at a flow rate of 0.8 mL/min and the Column temperature was maintained at 25°C. UV detection was performed at 244 nm with a run time of 10min. The injection volume was 15 µl. Prior to the first injection;

the column was equilibrated for 25 min with the mobile phase flowing through the system. Using these analytical conditions, imiquimod was eluted for about 5.377 min.

Preparation of diluent

Diluent was prepared by mixing 0.1 N HCl and acetonitrile in the ratio7:3 (v/v).

Preparation of standard solutions

Accurately weighed about 50 mg of imiquimod standard was taken in a 50 mL volumetric flask. Dissolve it in HPLC grade water with two drops of HCl was added and mixture was dissolved by sonication and it was diluted up to mark with HPLC grade water. Further dilutions were to 100 mL with mobile phase.

Preparation of sample solution

Cream sample equivalent to 50 mg of imiquimod was weighed and taken in a 50 mL volumetric flask Dissolve it in HPLC grade water with two drops of HCl was added and mixture was sonicated for 40 min with intermittent shaking and then cooled at room temperature. The resulting solution was diluted with up to the mark. Further dilutions were to 100 mL with mobile phase.

METHOD VALIDATION

Specificity

Specificity of proposed method was determined by checking blank and placebo interference at the retention time of imiquimod peak. Identification of imiquimod peak in sample solution was confirmed by comparing retention time of imiquimod peak with retention time of standard solution of imiquimod. Also imiquimod peak was checked for peak purity using Photo diode array detector (PDA).

Linearity

Linearity of the method was evaluated by using 5 linearity solutions of different concentrations. Accurately measured aliquots of working standard were taken in five different 100 mL volumetric flask and diluted up to the mark with the diluent such that the final concentrations of imiquimod were 5 ng/mL, 10 ng/mL, 20 ng/mL, 40 ng/mL, 60 ng/mL, 80 ng/mL, 100 ng/mL 200 ng/mL, 400 g/mL, 600 ng/mL. A 15 µL aliquot of each linearity solution was injected in duplicate.

Accuracy

The accuracy of the method was determined by calculating recoveries of imiquimod by the standard addition method. Known amount of standard of imiquimod was spiked to placebo in three different levels (80%, 100% and 120% of sample concentration) and prepared three spiked samples of each level (Total 9 determinations as per ICH guideline.) These spiked samples were analyzed against working standard and the amount of imiquimod recovered in three different levels was calculated.

Instrumental precision

The instrumental precision was checked by injecting five replicates of standard solution containing Imiquimod (5 µg mL^{-1}) and calculated the percentage RSD of retention time and area responses of imiquimod.

Method Precision (Repeatability)

The method precision of the proposed method was determined by preparing six different sample solutions of same batch and analyzed against working standard solutions. Assay values of these all six samples were calculated.

Intermediate precision (reproducibility)

The intermediate precision of the proposed method was evaluated by preparing six different sample solutions of same concentrations as prepared in method precision and analyzed against working standard solutions on different days. Assay values of all the six samples were calculated.

Robustness

Robustness of method is its ability to remain unaffected by small changes in method parameters. Robustness of proposed method was demonstrated by making slight changes in method parameters like flow rate (±5%), column temperature (±2°C), detection wavelength (±5 nm), mobile phase composition (±5% organic phase) and used different lot of column.

Filter compatibility

To check the compatibility of filter paper used to filter sample solution, the sample solution was divided into two parts. One part of solution was centrifuged and other part of solution was filtered through different types of filter papers such as 0.45 µm PTFE syringe filter, 0.45 µm PVDF filter and 0.45 µm Teflon syringe filter. Results of centrifuged sample and filtered samples were compared.

Solution stability

The solution stability of sample solution and standard solution were evaluated by comparison of assay value of freshly prepared samples and stored samples (at room temperature for 24 h). Standard solution and sample solution were prepared as mentioned in chromatographic conditions. Sample solution was analyzed and assay value was calculated against standard solution. Both the solutions (standard and sample solution) were kept at room temperature for 24 h. After 24 h these stored samples were reanalyzed against freshly prepared standard solution and the assay values were compared.

RESULTS AND DISCUSSION

In this method to optimize chromatographic parameters several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was obtained with

mobile phase consisting a mixture pH 4.6 phosphate buffer and Acetonitrile (20:80) v/v Since imiquimod having – NH_2 group, buffer pH in mobile phase plays vital role to achieve good peak symmetry of analyte. The proposed method gives very sharp peak shape of imiquimod with asymmetry factor less than 1.2 and theoretical plates above 2500. Analysis was carried out at wavelength 244 nm. Retention time of imiquimod is 5.773 min. The proposed method was validated as per ICH guidelines with respect to specificity, linearity, accuracy, precision, robustness, solution stability and filter paper compatibility. All results of validation parameters meet the limits of ICH guidelines. chromatogram of imiquimod, blank and placebo of imiquimod cream is shown in (Figure 2)

Specificity

It was observed that there was no interference from blank and placebo at the retention time of Imiquimod peak. Retention time of imiquimod peak in sample solution matches the retention time of imiquimod peak in standard solution. Also 3-point peak purity of imiquimod peak was 1.000. These results indicate that proposed method gives uniform and pure peak of imiquimod.

Linearity

A calibration curve was obtained by plotting area response versus concentration. Correlation coefficient obtained from graph was 0.992. Linearity curve of imiquimod is shown in (Figure 3)

Accuracy

The percentage recoveries of imiquimod from cream samples were calculated. Recovery ranged between 98.0% and 100.0%. Results of recovery experiment are shown in (Table 1)

Instrumental precision

The percent relative standard deviation (RSD) for five replicate of standard solution was found to be 0.50% and 0.26% for retention time and area response respectively.

Method precision

Percent relative standard deviation (RSD) of Assay values for six samples were found to be 0.16%. The low RSD values indicate that the proposed method is precise or repeatable.

Intermediate precision or reproducibility

%RSD of assay values of 12 samples (method and intermediate precision sample) were found to be 0.47%. The closeness of assay results and percent RSD values indicate that the proposed method is reproducible.

Robustness

It was observed that by making changes in chromatographic parameters, absolute difference between percent assay under altered condition and mean percent assay obtained during repeatability was not more than 2.0%. %RSD of area response and retention time were below 1%. (Table 2).

Filter compatibility

The percent assay values were calculated for centrifuged and filtered samples. The results obtained using filter paper was compared with results obtained with centrifuged sample. Absolute difference between results for filtered solutions and centrifuged solutions was not more than 2.0%. It was observed that filter paper does not adsorb drug substance during filtration of sample solutions.

Solution stability

Absolute difference between all assay values for freshly prepared and stored sample solutions at room temperature for 24 h was not more than 2.0%. The study shows that solution was stable up to 24 h.

Application of the developed method

The proposed method was applied for determination of content of imiquimod in the marketed samples of Imiquimod cream. Imiquimod cream samples from different manufacturers were purchased from market and analyzed for the amount of imiquimod using this proposed method. Results of analysis matched with percent label claim of marketed creams.

Conclusion

Literature survey reveals that there is no method reported for determination of imiquimod content from Imiquimod cream using reverse phase HPLC. Retention time of Imiquimod is about 5.773 min and total run time is 10 min. Very few methods are reported for imiquimod API and some biological samples but no any method reported for topical preparation (cream samples). The proposed method was found accurate, simple, precise, rapid and economical. Method validation parameters meet the specifications laid down in ICH guidelines. Hence, the method can be easily and conveniently adopted for routine analysis of imiquimod content in imiquimod cream.

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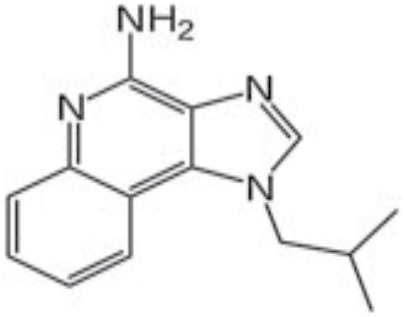

Figure 1. Structure of imiquimod.

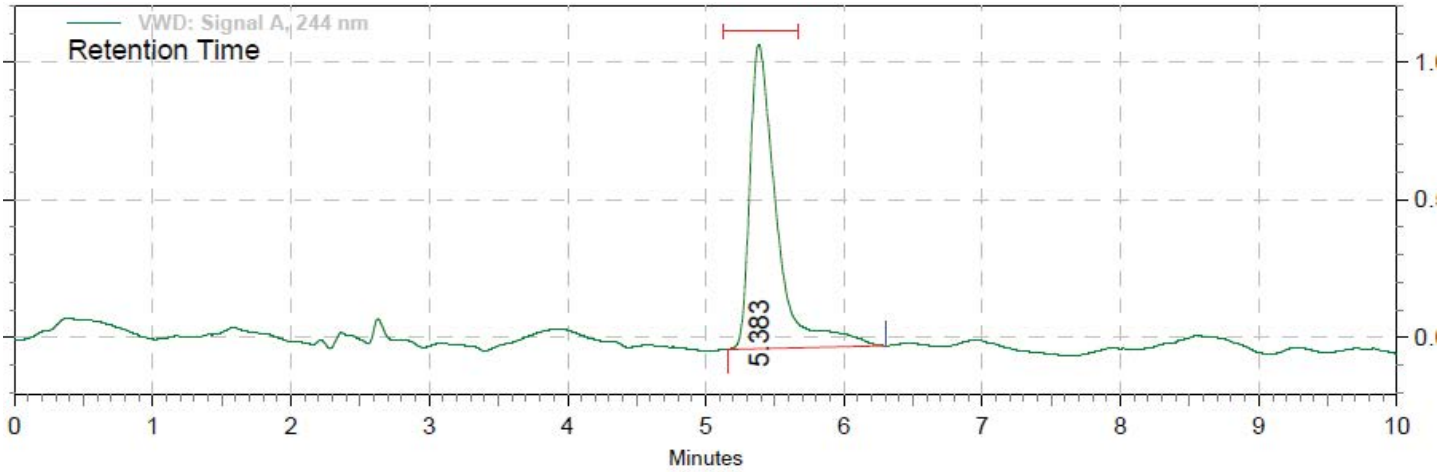

Figure 2. A typical chromatogram of Imiquimod.

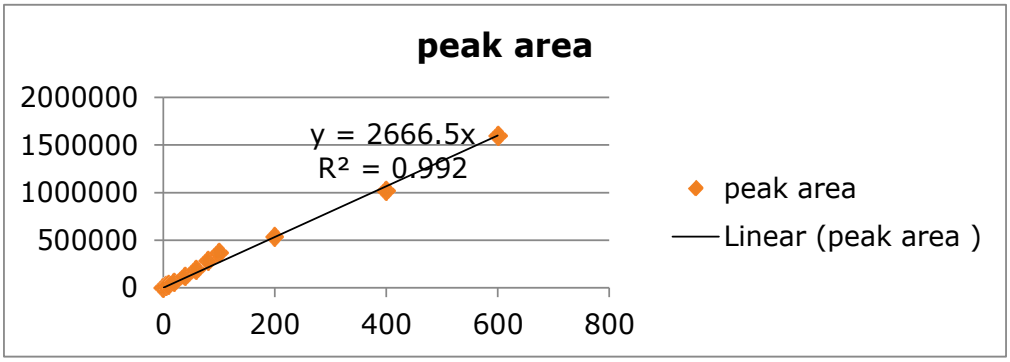

Figure 3. Linearity curve of imiquimod.

Table 1. Recovery results of imiquimod.

S.No.	Accuracy level	Sample preparations	Added amount of imiquimod (mg mL^{-1})	Recovered amount of imiquimod (mg mL^{-1})	%Recovery	Mean % recover	%RSD
1	Accuracy (80%)	Preparation-1	0.0100	0.0099	99.00	98.67	0.70
		Preparation-2	0.0100	0.0099	99.00		
		Preparation-3	0.0101	0.0099	98.02		
2	Accuracy (100%)	Preparation-1	0.0126	0.0125	99.21	99.47	0.56
		Preparation-2	0.0126	0.0125	99.21		
		Preparation-3	0.0125	0.0125	100.00		
3	Accuracy (120%)	Preparation-1	0.0152	0.0150	98.68	98.90	0.47
		Preparation-2	0.0151	0.0150	99.34		
		Preparation-3	0.0152	0.0150	98.68		

Table 2. Results of robustness study.

Method parameter	Altered condition	%Assay	%RSD
Flow rate	1.30 mL min^{-1}	98.5	0.71
	1.40 mL min^{-1}	98.8	0.14
	1.50 mL min^{-1}	99.0	0.35
Temperature	38 °C	98.2	0.12
	40 °C	98.8	0.14
	42 °C	99.1	0.09
Wavelength (nm)	240 nm	98.1	0.45
	245 nm	98.8	0.14
	244 nm	99.2	0.41
Mobile phase composition (Buffer:Acetonitrile v/v)	68:32	98.3	0.62
	20:80	98.8	0.14
	30:70	90.8	0.37
Columns	Lot-1	98.8	0.14
	Lot-2	98.7	0.06