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Original Article

A rapid and validated reverse phase liquid chromatographic method for determination of imiquimod from topical cream formulations

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ABSTRACT

A Novel, rapid, accurate and precise reverse phase isocratic HPLC method has been developed for the determination of Imiquimod content in Imiquimod cream which is widely used for treatment of precancerous skin lesions known as actinic keratosis, a certain type of skin cancer and treatment of genital warts, known as Human Papilloma Virus (HPV). A mixture of 10 mM monobasic phosphate containing 0.1% triethylamine adjusted to pH 2.45 and acetonitrile in the ratio of 70:30 (v/v) was used as mobile phase. C-18 column of dimension 250 × 4.6 mm, 5 μ from Inertsil was used for analysis. Detector wavelength was kept at 245 nm and flow rate was kept 1.4 mL min^{-1}. The retention time was observed at about 3.0 ± 0.1 min. The method has been validated as per ICH guidelines, with respect to specificity, linearity, accuracy, precision, robustness, solution stability and filter compatibility. Results for all the parameters were found within predetermined limits. The proposed method was applied to determine the content of Imiquimod in Imiquimod creams from different manufacturers. Content of the imiquimod was in good agreement with label claim of Imiquimod creams. This showed that proposed method is rapid, simple, precise, linear, robust, rugged and accurate which is useful and economic for routine analysis of Imiquimod content in Imiquimod cream.

1. 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.[7–9] 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).[10] It is also used as

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a treatment of precancerous skin lesions, known as actinic keratosis.[10] 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 Fig. 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.

2. Experimental

2.1. Chemicals and reagents

Imiquimod standard and cream samples were obtained as a gift samples from Cipla Limited. Ortho phosphoric acid (GR grade), triethyl amine (GR Grade), potassium dihydrogen phosphate and 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.

2.2. Chromatographic conditions

Mobile phase was a mixture of 10 mM monobasic phosphate containing 0.1% triethylamine adjusted to pH 2.45 with ortho phosphoric acid and acetonitrile in ratio of 70:30 v/v. Mobile phase was filtered through a 0.45 μm nylon filter and degassed for 5 min using an ultrasonicator. Mobile phase was pumped through the column at a flow rate of 1.4 mL min^{-1}. Analyses were carried out at 40 °C temperature and eluents were monitored at detection wavelength of 245 nm. The total run time was set as 5 min. The injection volume was 20 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 3.0 min.

2.2.1. Preparation of diluent

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

2.2.2. Preparation of standard solutions

Accurately weighed about 50 mg of imiquimod standard was taken in a 200 mL volumetric flask. About 150 mL diluent was added and mixture was dissolved by sonication and it was diluted up to mark with diluents. 5 mL of this solution was further diluted to 100 mL with mobile phase.

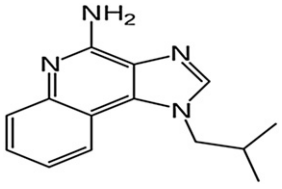

Fig. 1 – Structure of imiquimod.

2.2.3. Preparation of sample solution

Cream sample equivalent to 50 mg of imiquimod was weighed and taken in a 200 mL volumetric flask to which 150 mL of diluent was added and the mixture was sonicated for 40 min with intermittent shaking and then cooled at room temperature. The resulting solution was diluted with diluent up to the mark. 5 mL of this solution was further diluted to 100 mL with mobile phase. Filtered solution through 0.45 μm Teflon syringe filter.

2.3. Method validation

2.3.1. 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).

2.3.2. 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 10 μg mL^{-1}, 11.25 μg mL^{-1}, 12.50 μg mL^{-1}, 13.75 μg mL^{-1} and 15 μg mL^{-1}. A 20 μL aliquot of each linearity solution was injected in duplicate.

2.3.3. 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.

2.3.4. Instrumental precision

The instrumental precision was checked by injecting five replicates of standard solution containing Imiquimod (12.5 μg mL^{-1}) and calculated the percentage RSD of retention time and area responses of imiquimod.

2.3.5. 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.

2.3.6. Intermediate precision (reproducibility)

The intermediate precision of the proposed method was evaluated by preparing six different sample solutions of same