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Analytical Method Validation of Drug Release [Dissolution] by Revers Phase HPLC for Voriconazole

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ABSTRACT

Voriconazole is a second-generation azole antifungal agent indicated for use in the treatment of fungal infections including invasive aspergillosis, esophageal candidiasis, and serious fungal infections. Voriconazole works principally, by inhibition of fungal cytochrome P-450-mediated 14 alpha-lanosterol demethylation, an essential step in fungal ergosterol biosynthesis. Skin is one of the most readily accessible organs on the human body for topical administration and its the main route of topical drug delivery system. The intention of presenting in gel formulations is for better patient compliance and to reduce the dose of the drug and to avoid the side effects like liver damage and kidney damage associated with oral dosage forms. Voriconazole topical gel of carbopol based formulations was made. The main objective is to develop a method for determination of percentage drug release of Voriconazole Gel Formulations. The method, after development, is validated as per ICH guidelines Q2 (R1).

INTRODUCTION:

Transdermal drug delivery gives many important advantages such as it is easy for application, protect the active compound from gastric and enzymatic degradation, simple to terminate the therapy if the undesired side effects occurs[1]. Skin is a natural barrier, and only a few drugs can penetrate through it easily in sufficient quantity[2].

There are various skin infections caused by fungus. Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical routes. Antifungal works by exploiting differences between mammalian and fungal cells to kill the fungal organism without dangerous effect on the host. Voriconazole is an imidazole antifungal derivative and used for the treatment of local and systemic fungal infection. A wide variety of vehicles ranging from solid to semisolids and liquid preparations are available for topical treatment of dermatological disease as well as skin care. Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical route[3].

There are various medicated products that are applied to the skin. Such products are referred to as topical or dermatological products. There are various Hydrophilic polymers such as carbopol 940, hydroxyl propyl methyl cellulose (HPMC), Sodium alginate that is used in topical gel delivery system[4]. Based on molecular fraction these polymers are used concentration between 1-5 % in a topical formulation.

BRIEF INFORMATION ON GEL:

Gels for dermatological use have several favorable properties such as being thixotropic, greaseless, easily spreadable, easily removed, emollient, non-staining, compatible with several excipients and water-soluble or miscile[5-6]. The USP defines gel as a semisolid system consisting of dispersion made up of either small inorganic particles or large organic molecules enclosing an interpenetrated by a liquid. The inorganic particles form a three-dimensional structure. Gels consist of a two-phase system in which inorganic particles are not dissolved but merely dispersed throughout the continuous phase and large organic particles are dissolved into the continuous phase[7]. Fungal infections are very common and can be topical as well as systemic. Treatment of fungal infection includes medicines like Voriconazole, fluconazole, ketoconazole, clotrimazole, and grisofulvin[8-11].

The main objective is to develop a method for determination of percentage drug release of Voriconazole Gel Formulations. The method, after development, is validated as per ICH guidelinesQ2 (R1)[12].

Also, an objective of the study is to develop the analytical determination/parameters of drug release rather than the dissolution parameters.

Parameters considered for analytical method validation of drug release [dissolution] method for Voriconazole Gel Formulations.

- System suitability, Specificity
- Forced degradation
- Precision
- System precision
- Method precision
- Stability in the analytical solution
- Linearity
- Accuracy
- Range

EXPERIMENTAL

Table No.: 1 Optimized Chromatographic Conditions

Column	:	YMC Hydrosphere C18, 4.6 x 150 mm, 5µ or equivalent
Wavelength	:	252 nm
Injection volume	:	10 µL
Column Temperature	:	25°C
Sample try Temperature	:	25°C
Flow rate	:	1.0 mL/min
Run Time	:	10 minutes
Diluent	:	Mobile phase

a) Mobile Phase Preparation: Buffer:

Weigh and transfer 4.7g of monobasic sodium phosphate into l000 mL of water. Dissolve and mix.

b) Mobile Phase:

Mix 800 mL of buffer and 200 mL of Acetonitrile, Filter and degas by sonication.

c) Preparation of Standard

Weigh and transfer about 20 mg of Voriconazole standard into 50 mL volumetric flask, dilute to volume with diluent and mix well. Further, dilute 5.0 mL of this solution to 25 mL with diluent.

d) Preparation of Sample

Weighaccurately1 gm of gel andtransferinto200 ml amber color volumetric flask, add 150ml of diluent and sonicate it for 15 minutes; allow it to cool at room temperature.Makeupthevolumeuptothemarkwithdiluent.Dilute4mlofthissolutionto 50 ml with diluent.Filterthesamplethesamplesolutionthrough0.45µNylonfilter.

e) Procedure

Inject Diluent (one injection), then inject five replicate injections of standard preparation and check the system suitability parameters.

f) Calculation and Formulae:

For calculation and formulae, refer section 15.0

Calculate the % assay of Voriconazole by the following formula:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{V} \times \frac{P}{100} \times \frac{100}{LA}$$

Where,

AT : The average area of Voriconazole peak from the Sample chromatogram.

AS : The average area of the peak from the standard chromatogram

WS : The weight of Standard in mg.

DT : Dilution of the sample in mL.

DS : Dilution of standard in mL.

V : The volume of the sample taken (G)

P : % purity of standard

LA : Label Amount

SYSTEM SUITABILITY:

To verify the analytical system is working properly and can give accurate and precise results, the system suitability parameters are to be set.

Injected Diluent (one injection) and Standard preparation (5 injections), recorded chromatograms and checked the system suitability parameters.

Table No. 2: Details of System Suitability

Acceptance criteria	Results
The % RSD for the area of Voriconazole replicate injections of standard preparation should be NMT 2.0.	0.1
Theoretical plates for Voriconazole peak should be NL T 1500.	10286
Tailing factor for peak should be NMT 2.0	1.2

Data Interpretation:

From the above results, it can be concluded that the system is suitable for analytical method validation.

SPECIFICITY:

Specificity is the ability of an analytical method to assess unequivocally the analyte in the presence of component that may be expected to be present, such as impurities, degradation products, and matrix components.

Performed the specificity parameter of the method by injecting Diluent, Standard preparation, Sample preparation> Placebo preparation, Known impurities, and Sample spiked with impurities into the Chromatographic system and recorded the Retention times.

Acceptance Criteria:

Diluent, placebo and impurities peaks should not interfere with Voriconazole peak.

The peaks of Impurities and Voriconazole should not interfere with each other.

PRECISION:

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogeneous sample. The precision of the analytical method is usually expressed as the standard deviation or relative standard deviation (Coefficient of variation) of a series of measurements.

SYSTEM PRECISION:

A single injection of Blank (Diluent) and five replicate injections of standard solution were made on the system. Refer to the below Table. All the data were acceptable as per the system suitability requirements.

A single injection of Blank (Diluent) and five replicate injections of standard solution were made on the system. All the data were acceptable as per the system suitability requirements.

Table No.: 3 Method Precision

Table No.	% Release
1	98
2	99
3	100
4	98
5	97
6	98
Mean	**98.3**
SD	**1.835**
%RSD	**1.82**

METHOD PRECISION:

In method precision, a homogeneous sample of a single batch should be analyzed six times. This indicates whether a method is giving consisting results of a single batch. Analyzed Six independent sample solutions prepared and injected on the HPLC. The data shows that %RSD is within the acceptance criteria.

LINEARITY:

The linearity of an analytical method is its ability to elicit test results that are directly or by a well defined mathematical transformation, proportional to the concentration of an analyte in samples within a given range.

The Linearity of response was determined by preparing different concentrations of standard stock solution ranging from10 % to150% of the working concentration. The data is summarized in the below Table. The data shows that the response is found to be linear; the

Correlation coefficient is more than 0.999. TheY-intercept is also within the set criterion. The graphical depiction is included in the below Figure.

Table No.: 4 Linearity Details

Level	Concentration in ppm	Area Response
0	0	0
1	0.0205	422
2	0.0410	1230
3	0.0616	1866
4	0.0821	2436
5	0.1026	3119
6	0.1231	3899
7	0.1539	4797
8	0.2052	6475
9	0.4104	12935
10	0.8208	26007
11	1.3133	42925
12	1.6416	53968
13	1.9700	64841
14	2.4624	79661
15	3.2833	105596
16	4.1041	133281
17	4.9249	160631
Correlation coefficient		**1.000**
Regression coefficient		**1.000**
Slope		**32552.249**
Intercept		**-165.105**
% Intercept		**-0.3**

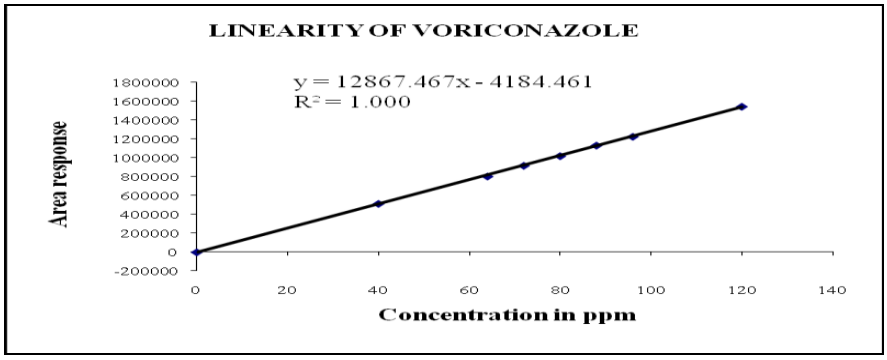

Figure No.1: Graphical Representation of Linearity of Voriconazole

ACCURACY:

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value (standard value).

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A spiked known quantity of Voriconazole standard at 50%, 80%, 100%, 120% and 150% of Assay specification limits into the placebo.

Performed precision at the lowest and the highest levels and for the other levels prepared in triplicate and injected in duplicate for all levels.

Calculated the % recovery from the results of Accuracy.

Table No.: 5 Recovery of Voriconazole.

% Level (about)	Sample	Mean Area Response	*mg/mL Added	*mg/mL Recovered	% Recovery	Mean % Recovery	%RSD
50	1	1240140	0.0392	0.0384	98.0	98.0	0.1
	2	1240095		0.0384	98.0		
	3	1243129		0.0385	98.2		
	4	1239626		0.0384	98.0		
	5	1239616		0.0384	98.0		
	6	1240746		0.0384	98.0		
100	1	2505096	0.0784	0.0776	99.0	98.9	0.2
	2	2501077		0.0774	98.7		
	3	2502763		0.0775	98.9		
150	1	3806360	0.1176	0.1178	100.2	100.4	0.1
	2	3815696		0.1181	100.4		
	3	3821258		0.1183	100.6		
	4	3811428		0.1180	100.3		
	5	3817426		0.1182	100.5		
	6	3811600		0.1180	100.3		

Data Interpretation:

From the above results, it can be concluded that the recovery is well within the limit. Hence the Method is accurate.

RANGE:

The range of analytical method js the interval between the upper and lower levels of analyte that has been demonstrated to be determined with suitable accuracy and linearity. Derived the specified range from the Linearity and Accuracy studies.

Acceptance criteria:

The % RSD obtained for all accuracy level determinations should be NMT 2.0.

The Correlation coefficient should be NL T 0.998 for Linearity and Accuracy level determinations.

RESULTS:

Table No.: 6 Linearity Range of Voriconazole

% Level	Concentration in ppm	Mean Area response
50	39.2117	1243904
100	80.3839	2511890
150	119.5955	3824363
	Correlation coefficient	1.000

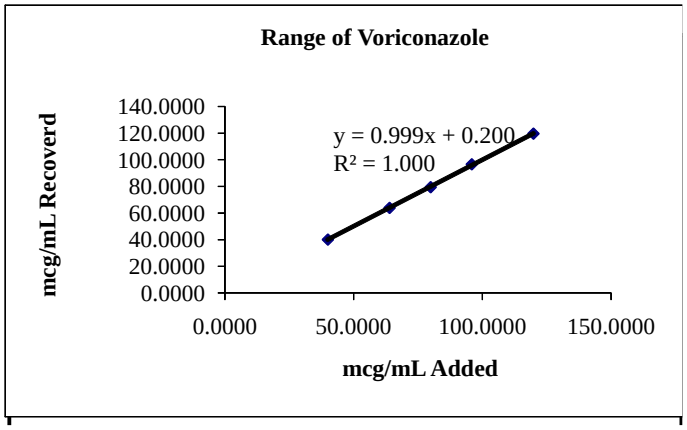

Fig No. 2 Graphical Representation of Accuracy Range of Voriconazole

ROBUSTNESS:

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Robustness Parameters:

a) Change in column oven temperature ± 5°C.

b) Change in flow rate ± 0.2 mL/min.

c) Change the organic phase ratio± 5.0 %

Acceptance criteria:

The System suitability parameters should pass for all the conditions.

Table No. 7 Robustness Parameter Details

System suitability Parameter		%RSD for Area	Theoretical plates	Tailing factor
Limit		NMT 2.0	NLT 1500	NMT 2.0
Original conditions		0.1	10286	1.2
Flow rate	1.2 mL/min	0.0	9213	1.2
	0.8 mL/min	0.1	11423	1.2
Column Temperature	30°C	0.1	10795	1.2
	20°C	0.1	9680	1.2
Organic Phase	+5%	0.2	9938	1.2
	-5%	0.1	10065	1.2

Data Interpretation: From the above results, it can be concluded that the Method is robust.

CONCLUSION:

The proposed HPLC method for estimation of Voriconazole in the Gel formulation by dissolution test methodology is validated. The method is found to be specific. The method is also stability indicating as evidenced by forced degradation studies. The method is found to be linear in the specified range for Voriconazole. The accuracy of this method is established. The method is found to be precise and robust. A system suitability test is established and related parameters are recorded. Hence this method stands validated and can be used for routine and stability analysis.

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