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DEVELOPMENT OF STABILITY-INDICATING HPTLC METHOD FOR TRIFLURIDINE

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ABSTRACT

Trifluridine is anantiviral agent widely used in the treatment of eye infection caused by *Herpes simplex* virus. The work involves the development of the stability-indicating HPTLC method for the determination of Trifluridine. A rapid and simple stability-indicating HPTLC method has been developed and validated according to ICHQ2(R1) guidelines. TLC aluminum plate precoated with silica gel 60F_{254} was used as a stationary phase. The solvent system used, consisted of methanol: chloroform(1.5 : 8.5 v/v). A compact band of the drug was observed at Rf0.42 ±0.02.Stress degradation studies were performed under various conditions viz. hydrolysis at different pH, oxidation, thermal and photolysis conditions as per ICH Q1A(R2) and Q1B guidelines. Densitometric scanning-integration was performed by CAMAG Scanner 3at 261 nm using winCATS Software.

KEYWORDS: HPTLC, Degradation, stability-indicating, Trifluridine.

INTRODUCTION

Trifluridine is also known as Trifluorothymidine. Chemically it is known as 5-Carboxy-2-deoxyuridine, which is freely soluble in water.[1] Herpes simplex virus encephalitis is devastating disease that is notoriously difficult to diagnose and has mortality rate over 70% if untreated[2], HSK is almost unilateral but can be severe with 10% of affected eyes losing vision below 20/100 after 20 years of follow up HSK is considered the leading infections cause of acquired blindness in western countries.[3] Trifluridine is an important antiviral agent for topical use in the treatment of deep surface herpetic infections, it involves inhibition of viral replication by incorporating into DNA during replication which leads to formation of defective protein by inhibiting thymidylate synthase,[4] and increased mutation rate. Trifluridine used in the treatment of keratoconjunctivitis and recurrent epithelial keratitis.

According to the literature survey[5-7], there is reported work on determination of Trifluridine in eye by HPLC method. But there is no stability-indicating HPTLC method developed for Trifluridine. The stress degradation study can be useful to predict the stability of the drug, possible degradation products formed when it is exposed to stress conditions. It helps to formulate, handle and store dosage form in a way to minimize degradation.

MATERIALS AND METHOD

Chemicals and Reagents

The working standard of Trifluridine was obtained from NATCO Pharma Hyderabad as a gift sample. Chemicals viz. methanol(HPLC grade), chloroform(HPLC grade) and glacial acetic acid (AR grade) were procured from LOBA CHEME Pvt Ltd.

Instrumentation

HPTLC system (CAMAG) was used. Aluminum plates pre-coated with silica gel 60 F_{254} (20 cm×20 cm) of 250 µm thickness (E. Merck, Darmstadt, Germany), Linomat-5 applicator (CAMAG), 100 µL microsyringe (Hamilton, Bonaduz, Switzerland), CAMAGTLC scanner-3 (CAMAG, Muttenz, Switzerland) with winCATS software version 1.4.3, NEWTRONIC® photostability chamber and JascoV-730 UV-visible spectrophotometer was used.

Chromatographic conditions

Different amounts of the working standard were spotted in the form of bands; bandwidth was 8 mm on precoated silica gel 60 F_{254} aluminum plates using 100µL Hamilton microsyringe on a CAMAG Linomat-5 sample applicator. Linear ascending development was done in a twin-trough glass chamber(CAMAG Muttenz)10×10 cm. The optimized mobile phase was methanol:chloroform (1.5: 8.5, v/v). TLC plates were air-dried. Densitometric scanning was performed using a CAMAG TLC scanner3 at 261 nm with winCATS software version 1.4.3.

Preparation of standard stock solution

The working standard solution was prepared by dissolving accurately weighed working standard Trifluridine in HPLC grade methanol, and making up the

volume of methanol to obtain a standard solution (200µg/ml).

Procedure

Forced degradation[9-10]

The study was performed as per the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q1A (R2) guidelines. The strength of stress reagent, exposure time were optimized to get 10-30% degradation.

Hydrolysis at acidic pH

For acid degradation, 200µg/mL of Trifluridine solution was prepared in methanol, 1 mL from this and 1 mL of 0.5 N HCl was taken and volume was made up to mark 10 mL using methanol. It was kept at room temperature for 1 h then HPTLC analysis was performed.

Hydrolysis at basic pH

The sample was prepared by adding 1 mL of solution(200µg/mL) to 1 mL of 0.1 N NaOH solution was made by methanol in a 10 mL volumetric flask. The sample solution was kept at room temperature for 1h then HPTLC analysis was performed.

Oxidative Degradation study

For oxidative degradation, 1 mL of standard solution (200µg/mL) was added to 1mL of 3% w/v H_2O_2 solution was prepared in a10 mL volumetric flask, volume made up to the mark by methanol and kept for 45 min at room temperature, then HPTLC analysis was performed.

Thermal degradation study

For thermal degradation, the standard drug was kept in a hot air oven at 45°C for 3 h, from this further dilution was prepared to obtained the concentration of 20 µg/mL.

Photolytic degradation study

For photolytic degradation, the working standard was kept in NEWTRONIC® photostability chamber under UV light for illumination upto 200-watt hrs/m^2 and white fluorescent lamp for not less than 1.2 million lux hours.

Method validation[8]

The developed method was validated as per the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use(ICH) Q2(R1) guidelines.

Specificity

For the specificity analysis of method, a standard stock solution was spotted and compared to the placebo spot of marketed formulation. The peak purity values of stress samples were used to check the specificity of the method.

Linearity

A standard stock solution of 20µg/mL was applied on a TLC plate within the range (5µL-25µL) to obtain 100-500ng/band in five replicates. The peak area versus amount spotted was evaluated by linear regression analysis. The graph is shown in Fig. 2.

Accuracy

A recovery study was carried out at three levels, 80%, 100% and 120%, to check the accuracy of the method. As the marketed sample was not available, a premix of common excipients and drug was prepared as per the label claim of VIROPTIC®1% eye drops.

Precision

The precision was evaluated by repeatability and intermediate precision studies. Intra-day precision was checked by performing three replicates of three different concentrations on the same day (morning, afternoon, evening) and inter-day precision was done by performing the study in triplicate on three different days.

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ of the developed method were calculated from the standard deviation of the lowest response and slope of the calibration curve as per the ICH guidelines.

Limit of detection = $3.3 \times \sigma/S$

Limit of quantification = $10 \times \sigma/S$

where σ is the standard deviation of response, S is the slope of the calibration curve.

Robustness

The robustness was performed by small, deliberate changes in scanning wavelength, chamber saturation time, change in time from spotting to development and change in time from development to scanning. The impact on the peak area was noted.

RESULT AND DISCUSSION

Optimization of HPTLC method

Neat solvents were used to check suitable solvents for the mobile phase composition. To optimize mobile phase various solvent composition were tried such as, ethyl acetate:toluene(5:5 v/v), methanol: chloroform(5:5v/v), methanol:chloroform (3 :7v/v).The optimized mobile phase was methanol:chloroform (1.5 : 8.5 v/v), in which an acceptable retardation factor and peak shape was found.

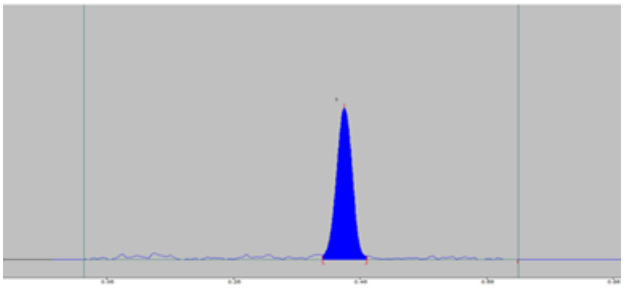

Fig. 1: Densitogram of Trifluridine working standard (100 ng/band).

Forced degradation study

Forced degradation at different conditions was performed. The result obtained is mentioned in Table1. In all stress conditions, there was decrease in the peak area of the stress degraded sample as compared to the peak area of the working standard solution. But there was no degradation product found in any of the stress condition. In photolytic degradation the working standard was found to be non-degraded.

Table 1: Forced degradation studies of Trifluridine.

Type of degradation	Temperature and time	% Recovery	r(s, m)	r(m, e)
Acid degradation (0.5 N HCl)	RT for 1 h	84%	0.9958	0.9998
Base degradation (0.1 N NaOH)	RT for 1 h	89%	0.9957	0.9969
Oxidative degradation (3% H_2O_2)	RT for 45 min	85%	0.9994	0.9970
Thermal degradation	At 45°C for 3 h	87%	0.9994	0.9958
Photolytic degradation	RT	100%	0.9995	0.9976

METHOD VALIDATION

Specificity

Specificity was evaluated by comparing the standard stock to the placebo spot of the marketed formulation. It was found to be there is no interference of any other substance at the Rf of the working standard. Peak purity values for all stressed sample confirms specificity.

Linearity and range

The linearity range was selected as100-500 ng/band. When amount spotted was plotted against peak area, the linearity equation was found to be y = 5.897x + 388.1. The correlation coefficient (R^2) 0.9919. Shows there is a good correlation between peak area and the amount of drug spotted. As shown in Fig.2. The 3D densitogram of linearity as shown in Fig. 4.

Testing the residual is one of the simplest linearity tests suggested for HPTLC method. The above plot shows no tendency behavior and thus linearity of calibration curve. Residual plot is as shown in Fig. 3.[11-12]

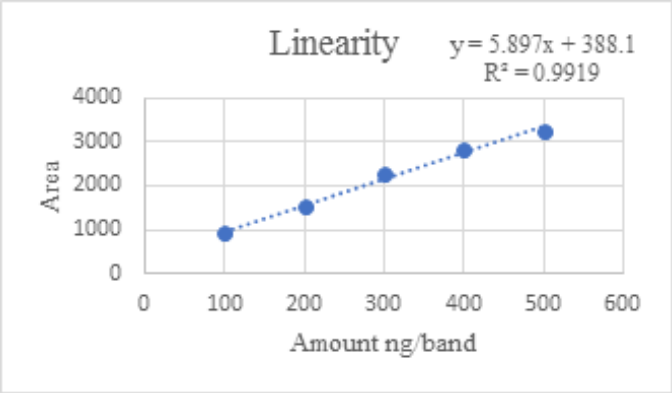

Fig. 2: Linearity graph plot of amount spotted versus peak area.

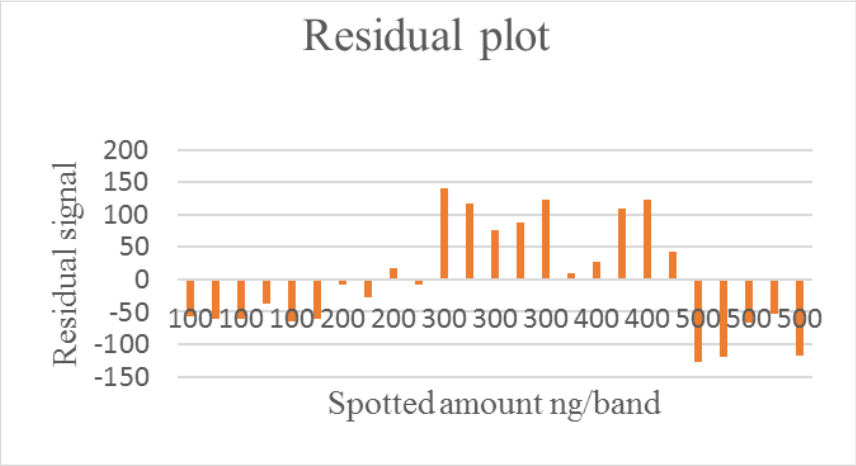

Fig. 3: Residuals plot of residual signal versus spotted amount ng/band.

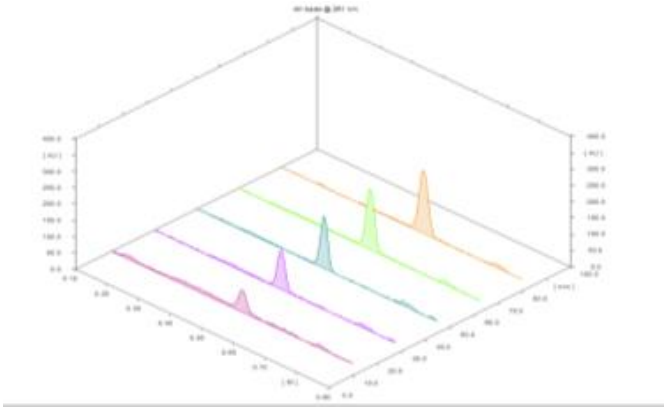

Fig. 4: Densitogram of linearity of Trifluridine 100-500 ng/band.

Accuracy

For accuracy, % recovery was obtained in the range of 98% to 102%. This proves the method to be accurate.

Precision

Calculated % RSD for the concentrations were found within the limit (<2%). This shows the developed method is precise, as it satisfies the acceptance criteria for the %RSD value. The result is shown in Table2.

Limit of detection and Limit of quantitation

The determination of LOD and LOQ was based on the standard deviation of the response of the calibration curve. The slope of the calibration curve was used to calculate LOD and LOQ. For the developed method, LOD and LOQ were found to be as follows, indicating that the developed method is sensitive.

LOD- 6.10 ng/band

LOQ- 18.49 ng/band

Robustness

For robustness, it was ascertained that deliberate change in saturation time, change in time from spotting to development, change in time from developed plate to scanning and change in wavelength did not affect the peak area of the working standard solution. The %RSD was found to be within the limit as shown in Table 2.

Table 2: Validation parameters of Trifluridine.

Sr No.	Validation parameter		Result
1	Linearity		y = 5.897x + 388.1 R^2= 0.9919
2	Range		100-500 ng/band
3	Accuracy	80%	101.79
		100%	102.86
		120%	103.98
4	Precision	Same day	1.36
	intermediate (%RSD)	Different days	1.14

5	LOD		6.10 ng/band
6	LOQ		18.49 ng/band
7	Robustness	Saturation time ±5 min	1.66
	(%RSD)	Mobile phase composition	1.46
		Mobile phase volume	1.73
		Development time 0, 5& 10 min	1.74
		Scanning time 0, 5 & 10 min	1.64
		Wavelength ±1nm	1.85
8	Specificity		Specific

DISCUSSION

To the best of our knowledge, there is no published report on the stability-indicating HPTLC method for Trifluridine. This study is the first where the stress degradation behavior of Trifluridine was evaluated by the HPTLC method. For all conditions under which degradation was carried out, it was found that there is no degradation product under any stress conditions.

Validation of optimized HPTLC method was carried out as per the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH)guidelines. All the validation parameters showed results within the limit. Multiwavelength scanning was performed to check the degradation product as shown in Fig. 5

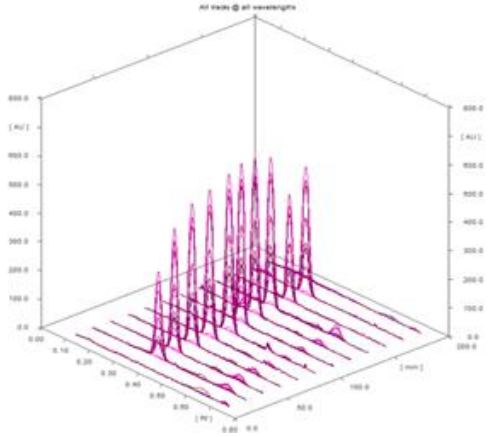

Fig. 5: 3D densitogram of multiwavelength scanning of linearity(2-6) and all degradation spot.[7-11]

CONCLUSION

The developed method was validated as per International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines and found to be rapid, economic. The method shows reproducible results, it can be used to monitorthe stability of Trifluridine.

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Conflict of interest

The authors declare that they have no conflict of interest.

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