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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR THE ESTIMATION OF VORTIOXETINE HYDROBROMIDE

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ABSTRACT

The present study describes the development of a simple, precise, accurate and specific validated HPTLC method for the estimation of Vortioxetine Hydrobromide. The separation was performed on TLC aluminium plates pre-coated with silica gel G60 F_{254} as the stationary phase. The method was developed at 230nm. The solvent system consisted of Chloroform: Methanol: Glacial acetic acid (92:8:5 v/v/v) provided optimum polarity for proper migration of Vortioxetine Hydrobromide and gave the R_f = 0.65±0.03. The proposed method was validated in terms of linearity, precision, accuracy, specificity and sensitivity as per the ICH guideline. The linearity was found to be within range 500-900 ng/spot with correlation coefficient was found to be 0.9932. The LOD and LOQ were found to be 37.81 ng/spot and 114.6 ng/spot for Vortioxetine Hydrobromide respectively.The %RSD was found to be <1 for intermediate precision and repeatability.

KEYWORDS: HPTLC, Vortioxetine Hydrobromide, Development, Validation.

INTRODUCTION

Depression is an etiologically heterogeneous group of brain disorder characterized by many symptoms which reflects alternations in cognitive, psychomotor and emotional processes. Sometimes depression comes with symptoms of anxiety. Depression is a common mental disorder with symptoms of depressed mood, loss of interest or pleasure, decreased energy, feelings of guilt or low self-worth, disturbed sleep or appetite [1,2,3].

Vortioxetine Hydrobromide (figure 1) is developed for the treatment of major depressive disorders by Lundbeck and Takeda Pharmaceutical Company Ltd. It was approved in the US under the trade name Brintellix in 30 September, 2013.Then approved by European Medicine Agency (EMA) on Dec 18, 2013[4, 5].

Vortioxetine was previously trademarked as Brintellix in the United States but on May 2, 2016, the US FDA approved a name change to Trintellix in order to avoid confusion with the blood-thinning medication Brilinta (Ticagrelor) [5,6].

Vortioxetine is classified as a serotonin modulator and simulator (SMS) as it has a multimodal mechanism of action towards the serotonin neurotransmitter system. Vortioxetine acts via biological mechanisms as a serotonin reuptake inhibitor (SRI) through inhibition of the serotonin transporter, as a partial agonist of the 5-HT_{1B} receptor, an agonist of 5-HT_{1A} and an antagonist of the 5-HT_3, 5-HT_{1D}, and 5-HT_7 receptors [7,8].

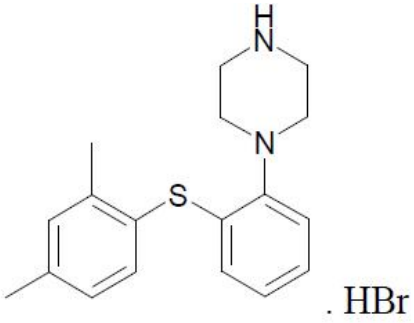

Figure 1: Structure of Vortioxetine Hydrobromide

Several analytical methods like RP-HPLC,UPLC-MS/MS, HILIC–MS have been reported for the estimation of Vortioxetine in bulk and human plasma and rat plasma. One RP-HPLC method has been reported for the estimation of impurities produced during the manufacturing of Vortioxetine.[9-14]

To the best of our knowledge, no HPTLC method has been reported for the estimation of vortioxetine Hydrobromide.So, the aim of present work was to develop the HPTLC method for estimation of Vortioxetine Hydrobromide and to validate developed method according to ICH guideline.[15]

MATERIALS AND METHODS

Materials and reagents:

Vortioxetine Hydrobromide standard was obtained as gift sample from Intas pharmaceutical (Ahmedabad, Gujarat). All reagents used were of analytical grade and purchased from CDH (P) Ltd. New Delhi, India. Precoated Silica gel G60 F_{254} HPTLC plates were purchased from Merck, Darmstadt, Germany.

Instruments

Chromatographic separation performed with CAMAG Linomat IV sample applicator equipped with Hamilton syringe and CAMAG TLC Scanner 3. CATS4 Software was used for data acquisition. A double beam UV-visible spectrophotometer (Shimadzu, model UV-1700) having two matched quartz cells with 1 cm light path was used for recording of spectra and measuring

absorbance. An electronic analytical weighing balance (Shimadzu AEG-220).

Optimized Chromatographic condition

HPTLC analysis was performed at aluminium backed HPTLC plates 20×20 cm coated with 0.2 mm layers of silica gel 60 F_{254} (Merck, Germany). Samples of 10µL were applied on plate with band width 5mm employing linomat IV sample applicator (Camag, Switzerland) fitted with microliter syringe. TLC plate was developed in ascending mode in twin trough chamber with mobile phase Chloroform: Methanol: Glacial acetic acid (92:8:5 v/v/v) upto 70 mm length. The plate was removed from the chamber, dried and analyzed at 230 nm using Camag TLC Scanner 3. Data of peak area was recorded using Camag CATS4 software. Various mobile phase were tried for analysis of Vortioxetine Hydrobromide and finally Chloroform: Methanol: Glacial acetic acid (92:8:5 v/v/v) at room temperature with 10 min saturation time provided optimum polarity for proper migration of Vortioxetine Hydrobromide and gave the R_f =0.65±0.03 which is summarized in Figure 2.

Preparation of standard solution of Vortioxetine Hydrobromide

Accurately weight Vortioxetine Hydrobromide (50 mg) was transferred to 50 mL volumetric flask, dissolved and diluted up to the mark with methanol (1000 µg/mL).10 mL from stock solution was pipetted out and transferred into 50 mL volumetric flask and diluted up to the mark with methanol (200 µg/mL).Aliquots of 2.5, 3, 3.5, 4, 4.5 mL were pipetted in the series and transferred to 10 mL volumetric flask and diluted up to mark with methanol (50-90 µg/mL).

Preparation of test solution of Vortioxetine Hydrobromide

Preparation of placebo solution:

Accurately weight excipients mannitol (140 mg), microcrystalline cellulose (20 mg), magnesium stearate (5mg), hydroxyl propyl cellulose (12mg), sodium starch glycolate (5 mg) and titanium dioxide (5 mg) were mixed uniformly and dissolved in methanol (100 mL) in 100 mL volumetric flask, sonicated for 10 minutes and filtered. The filtrate was used as the placebo solution.

Preparation of solution:

Stock solution of Vortioxetine Hydrobromide (S1): Vortioxetine Hydrobromide (10mg) was weighed accurately and transferred into 10 mL volumetric flask and diluted with methanol upto mark (S1=1000 µg/mL).

Working standard solution (S2): 2.5 mL was pipette out from stock solution and transferred into 25 mL volumetric flask and diluted up to the mark with methanol (S2=100 µg/mL).

Accuracy (80%): From the working standard stock (100 µg/mL) 4.8 mL was pipette out in a 10 mL volumetric flask and diluted upto the mark with placebo solution to make the final concentration 48 µg/mL.

Accuracy (100%): From the working standard stock (100 µg/mL) 6 mL was pipette out in a 10 mL volumetric flask and diluted upto the mark with placebo solution to make the final concentration 60 µg/mL.

Accuracy (120%): From the working standard stock (100 µg/mL) 7.2 mL was pipette out in a 10 mL volumetric flask and diluted upto the mark with placebo solution to make the final concentration 72 µg/mL.

RESULTS AND DISCUSSIONS

Validation of method

The method was validated according to the ICH guideline by determining Linearity and range, precision,Limit of detection (LOD),Limit of quantitation (LOQ),peak purity and accuracy and robustness.

Linearity and Range: Linear relationship was obtained between response (peak area) and concentration of Vortioxetine Hydrobromide in the range of 500-900 ng/spot at 230 nm. The linear response is shown in figure 3. The correlation coefficient was found to be 0.9932.

Precision: The precision expressed as standard deviation or relative standard deviation. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Repeatability: Repeatability of Sample application was assessed by amount of Vortioxetine Hydrobromide 700 ng spotted six times and scanned. %RSD was found to be 0.42% for Vortioxetine Hydrobromide and Repeatability of measurement of peak area was determined by Vortioxetine Hydrobromide 700 ng spotted and scanned for six times. % RSD was found to be 0.16%.

Intermediate precision:

Intraday precision: Precision was studied by analysing 3 concentrations (500,700,900 ng) on the same day. The %RSD for intraday precision was found to be 0.16-0.19% for Vortioxetine.

Inter-days precision: Precision was studied by analysing 3 concentrations (500,700,900 ng) on 3 consecutive days and %RSD for interday precision was found to be 0.10-0.47%.

Limit of detection and Limit of quantitation: LOD and LOQ were determined by standard deviation (SD) method from the slope (*S*) of calibration plot by use of the equations.

LOD=3.3×SD/S and LOQ=10×SD/*S*. The LOD and LOQ were found to be 37.81 ng/spot and 114.6 ng/spot for Vortioxetine Hydrobromide respectively.

Specificity: The purity of Vortioxetine was ascertained by peak purity spectrum at three levels peak start, peak apex and peak end. It shows correlation coefficient of 0.999850 (S, M) and 0.999870 (M, E).

Accuracy: Accuracy of method was tested by determination of recovery at three levels. Pre-analyzed samples were spiked with extra individual standards (80, 100 and 120). Each determination was performed in triplicate. The results of recovery have shown in table no 1.

Robustness: There was no significant change was observed in chromatographic pattern when the modifications were made in the experimental conditions, indicated that method was robust. Change in different parameters as detection wavelength (±1 nm), saturation time (±2 min) and run distance (±5 mm) were give %RSD <1.

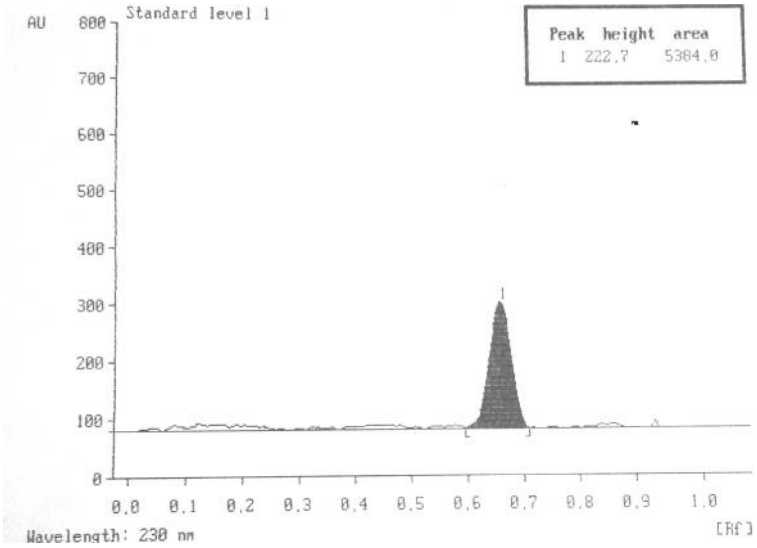

Figure 2: Chromatogram for standard Vortioxetine Hydrobromide (R_f =0.65±0.03)

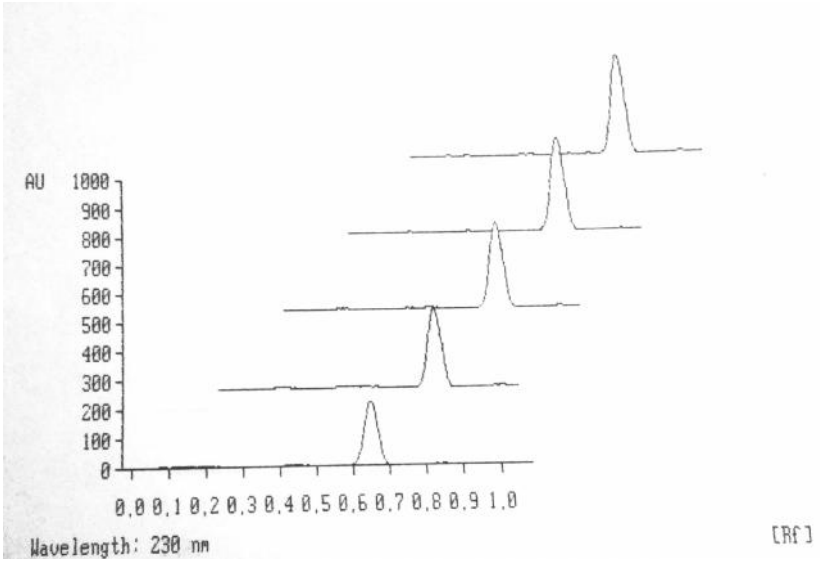

Figure 3: Chromatogram for calibration of Vortioxetine Hydrobromide

Table 1: Recovery study for Vortioxetine Hydrobromide

Level	**Amount spiked in placebo solution (µg/mL)**	**Target concentration in (ng/spot)**	**Amount of Vortioxetine recovered (ng/spot) Mean ± SD (n=3)**	**% Recovery Mean ± SD (n=3)**
80	48	480	479.17±6.65	99.83%
100	60	600	601.75±16.70	100.28%
120	72	720	713.66±14.87	99.12%
%Average Recovery ± SD=99.75%				

Table 2: Validation data of HPTLC method for estimation of Vortioxetine Hydrobromide

SR. NO.	PARAMETERS	RESULTS
1.	**Linearity range (ng/spot)**	0-900
2.	**Correlation coefficient**	0.9932
3.	**Precision** 1. **Intermediate precision(% RSD)** I. Intraday precision (n=3) II. Interday precision (n=3) 2. **Repeatability (%RSD)** I. Repeatability of measurement of peak area II. Repeatability of sample Application	 0.16-0.19 0.10-0.47 0.16 0.43
4.	**Limit of Detection (ng/spot)**	37.81
5.	**Limit of Quantification (ng/spot)**	114.6
6.	**Specificity**	Specific
7.	**Accuracy**	99.75%

CONCLUSION

The developed HPTLC method was found to be simple, less expensive, fast, accurate and precise so it can be used for routine analysis of Vortioxetine Hydrobromide in bulk and synthetic mixture.

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