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"Method Development and Validation Using HPTLC for Determination of Cariprazine in Pharmaceutical Dosage Form"

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ABSTRACT:
The selected drug Cariprazine is an atypical antipsychotic agent. It is used for the treatment of the schizophrenia and bipolar disorder. An extensive literature surveys reveals that few chromatographic (HPLC and LC/MS) and UV-spectrophotometric methods were reported for the estimation of Cariprazine in pharmaceutical dosage form. So, it was thought to developed simple, precise, accurate and specific HPTLC method for the estimation of Cariprazine in pharmaceutical dosage form. The separation was achieved on pre-coated silica gel aluminium plate 60 F254 as a stationary phase using ethyl acetate: methanol (7:3, v/v) as a mobile phase. The densitometric measurement of separated components was performed at 257 nm The method was validated for accuracy, precision, reproducibility, robustness, limit of detection and limit of quantification as per International Council for Harmonization [ICH Q2(R1)] guidelines. The regression coefficients (r2) was found to be 0.9979 and the values for percentage recoveries was found to be 99.66 – 101.55%. The developed HPTLC method can be used for the analysis of cariprazine in pharmaceutical dosage form.

KEYWORDS: Cariprazine, HPTLC, ICH guideline

AIM:Development and validation of HPTLC method for estimation of cariprazine in bulk and pharmaceutical dosage form.

OBJECTIVE:•To develop HPTLC method. • To validate the developed method as per ICH Q2R1 Guidelines. • To apply the developed method for the assay of the pure drug as well as in dosage form so that method will be applicable to routine analysis of the drug in QC labs in future

I. INTRODUCTION:

Cariprazine is a medication that treats mental health conditions like schizophrenia and bipolar disorder. It balances the levels of dopamine and serotonin in your brain. These substances help regulate your mood, behaviors and thoughts.

Literature survey reveals many analytical methods for its estimation. In the present investigation simple, accurate, sensitive and precise HPTLC method has been developed for determination of Cariprazine in the bulk and marketed formulation.

II. EXPERIMENTAL:

Drugs, Reagents and Chemicals used: Cariprazine was kindly gifted by Cadila Pharmaceuticals, Ahmedabad, India. Analytical grade toluene was procured from RFCL Ltd. New Delhi.AR grade methanol and ethyl acetate were procured from Loba Chemical Private Limited.

INSTRUMENTATION: HighPerformanceThin LayerChromatography (HPTLC) **Make:** Camag HPTLC System, Switzerland **Plate:** Pre-coated silica gel aluminium plate G 60 F254 **Size:**10×10mm, **Applicator:** Linomat V automatic sample applicator **Chamber:** Camag Twin through TLC developingchamber(10× 10mm)

Preparation of Standard Stock Solution:
After carefully weighing 10 mg of cariprazine, it was transferred to a 10 ml volumetric flask. The volume was made up to the mark using methanol to obtain a concentration of 1000 µg/ml.

Preparation of calibration curves:
To create the calibration curve, Hamilton micro syringes with Linomat V applicator were used to apply aliquots of the working standard solution, which had a concentration of 2000-6000 ng/band, to pre-coated silica gel G 60 F254 aluminium plates. Ethyl acetate: Methanol (7:3 v/v) mobile phase was used to develop the plate in a previously saturated chamber for 20 minutes and dried in the air. Developed plate subjected to densitometric measurement at 257 nm using the absorbance mode

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on a Camag TLC scanner. The peak area and drug concentration are then plotted on a graph.

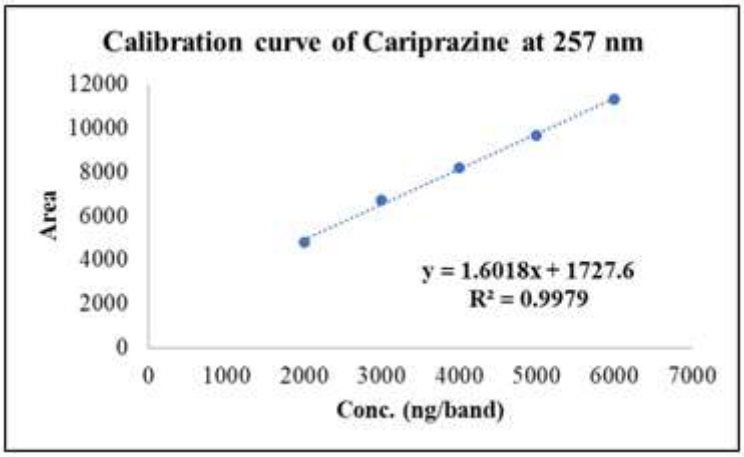

Figure 1: Calibration curve of Cariprazine at 257 nm

Analysis of capsule formulation:

Analysisof cariprazineinmarketedformulation

To determine the amount of cariprazinein the capsule10mgquantityofcapsulepowderwas addedtoa10mlvolumetric flaskfilled with 7 ml of methanol and sonicated for 15 minutes. Then it was filtered and dilutedto 10 ml with methanol to achieve 1000 µg/ml. On a TLC plate, 2µl of this solution was applied, and then it was developed and scanned at 257 nm. Three repetitions of the analysis were conducted.

METHOD VALIDATION: As per the ICH guidelines the method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantitation and robustness and specificity.

LINEARITY: By spotting 2, 3, 4, 5 and 6 µl of the standard solution containing 1000 µg/ml of CARI, the linearity was evaluated. Linearity of responses was evaluated in the 2000– 6000 ng/band concentration range. The mean peak area of cariprazine was calculated after six separate chromatographic runs of the sample solution.

ACCURACY:

For accuracy of method, recovery study was carried out by applying the method to drug sample to which known amount of cariprazine was added at level of 50, 100 and 150% of label claim (standard addition method). At each level of the amount, three determinations were performed and the results obtained were compared with expected results.

PRECISION:

Three different quantities of cariprazine 2000ng/band, 4000ng/band, and 6000 ng/band were used in replicate at three different times on the same day to accomplish intraday precision and triplicate on three different days 2000ng/band, 4000ng/band, and 6000ng/band inter-day precision was conducted.

SPECIFICITY:

The specificity of the method was ascertained by overlaying UV spectra of spots for standard drug and sample.

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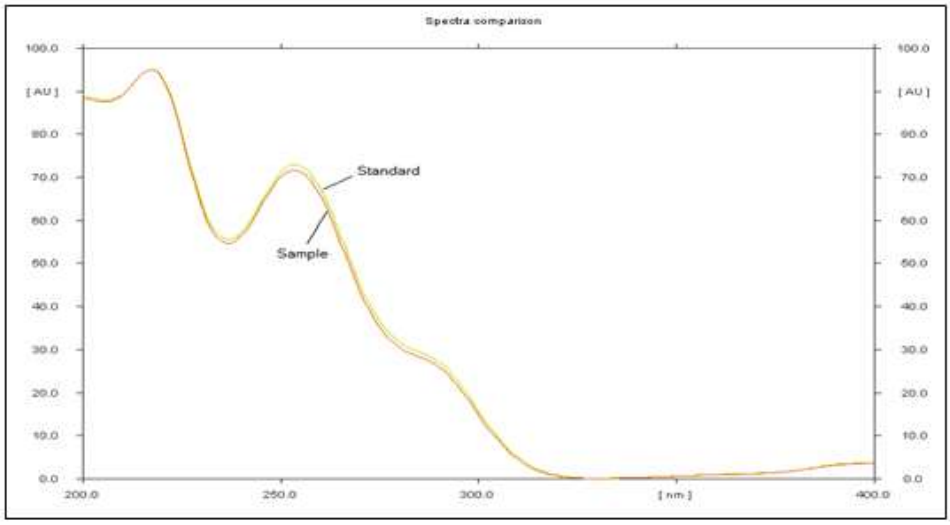

Figure2:OverlainspectraofstandardAPIandcapsuledosageform

ROBUSTNESS: Robustness is checked by making slight deliberate change in the experimental procedures. The influence of a slight, intentional change in the analytical conditions on the drug's peak areas was studied. The impact of altering the chamber saturation time (± 2 min) and detecting wavelength (± 2 nm) was investigated, and the percentage RSD was calculated.

Sr.No.	Parameter	Level	Meanarea±SD	%RSD
1	Chamber Saturationtime	25 min	8143.63 ± 41.50	0.510
2		30 min	8197.1 ± 50.50	0.616
3		35 min	8387.63 ± 68.89	0.821
1	Wavelength	252 nm	8150 ± 10.65	0.131
2		257 nm	8192.1 ± 7.46	0.091
3		262 nm	8225.33 ± 9.70	0.118

Table1: Results forrobustnessstudyforCARIPRAZINE

Limit of Detection and Limit of Quantification: The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOD and LOQ were calculated using the following formula.

$LOD=3.3\times\sigma/S$ $LOQ= 10 \times \sigma/S$

Where,σ =Standard Deviation of the y-intercept S = Slope of calibration curve

III. RESULT AND DISCUSSION

Optimization of Solvent System and Chromatographic Conditions:

Chromatographic separation studies were carried out on the stock solution of cariprazine. Initially the plates were spotted with 10 µL of stock solution and developed by linear ascending development method using neat solvents like toluene, methanol, ethyl acetate and ammonia without chamber saturation. Based on the results of these initial chromatograms, binary and ternary mixtures of solvents were tried to achieve optimum peak parameter. The final mobile phase consisting of Ethyl acetate: methanol in the ratio of (7: 3, v/v)was optimized since good Rf value of for cariprazine was obtained as shown in Fig. 4. The samples were applied in form of bands of width 6 mm on precoated aluminum sheets of silica gel 60

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F254. The application position (X) and (Y) were kept at 10 mm and 10 mm respectively to avoid edge effect. Linear ascending development was carried out in a twin trough glass chamber (20cm x 10 cm, 10 x 10 cm), using 15 mins of chamber saturation. Densitometric scanning was performed in the absorbance mode at 257 nm. The slit dimension was kept at 5 x 0.45 mm.

IV. CONCLUSION

The validated HPTLC method employed here proved to be simple, fast, accurate, precise and sensitive, thus can be used for routine analysis of cariprazine in capsule dosage form.

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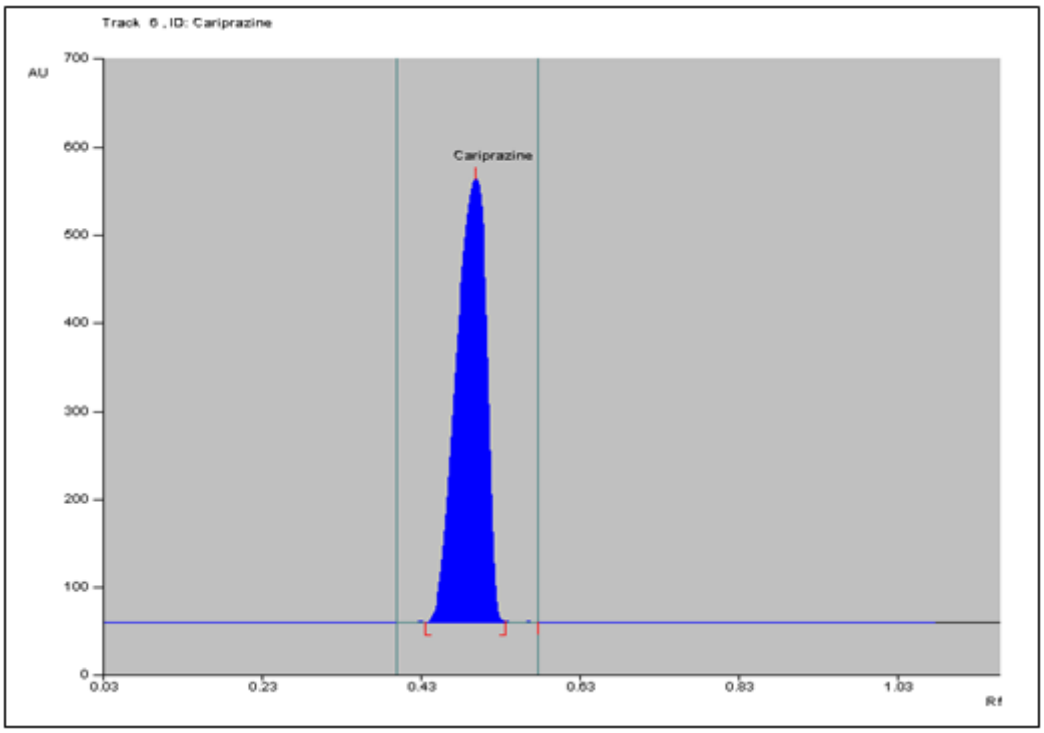

Figure3::HPTLC densitogram of Cariprazine

Table 2: DataofAccuracystudyforCARI

Sr. No	Level	Amt.present (ng/band)	Amt.ofstd. APIspiked (ng/band)	Totalconc. (ng/band)	Amt. found (ng/band)±SD	Amt.recovered (ng/band) ±SD	Mean%recovery ± SD	%RSD
1	0%	2000	0	2000	1985.24 ± 36.01	-	-	-
2	50%	2000	1000	3000	2990.39 ± 12.29	1015.49 ± 11.75	101.55 ± 1.18	0.18
3	100 %	2000	2000	4000	4011.88 ± 14.82	2022.21 ± 14.82	101.5 ± 0.74	0.78
4	150 %	2000	3000	5000	4979.42 ± 27.53	2989.75 ± 27.53	99.66 ± 0.91	0.42

(Average of three determination)

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Table 3:ResultsofLODand LOQforCARI

Sr.No.	Parameter	Concentration(ng/band)
1	LOD	104.54
2	LOQ	317.39

(Average of three determination)

Table 4: Assayresultsofcariprazineinpharmaceuticalformulation

Drug	Labelclaim (mg)	Amt.found(mg) ± SD	%Assay ± SD
Cariprazine	4.5	4.41 ± 0.3098	99.48 ± 1.05

(Average of three determination)

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