International Journal for Pharmaceutical Research Scholars (IJPRS)

V-5, I-2, 2016 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Development and Validation of Stability Indicating Analytical Method for the Determination of Cabergoline

Lasan VM*, Indrekar TD

Department of Quality Assurance, L. M. College of Pharmacy, Ahmedabad, Gujarat, India.

Manuscript No: IJPRS/V5/I2/00456, Received On: 03/05/2016, Accepted On: 08/05/2016

ABSTRACT

A simple reproducible and efficient high-performance thin layer chromatographic (HPTLC) method has been developed and validated for the stability study of cabergoline. The method was employed on TLC aluminium plates precoated with silica gel 60 GF_{254} as the stationary phase. The solvent system consisted of chloroform: methanol: ammonia (25%) (80:20:1). This system was found to give compact spot for Cabergoline (R_f value of 0.65-0.03). UV detection was performed at 280 nm. Cabergoline was subjected to acid, alkali, neutral hydrolysis, oxidation, thermal degradation, and photolytic degradation. The drug undergo degradation under acidic and basic conditions, and oxidative degradation. The method was validated for linearity, precision, limit of detection (LOD), limit of quantification (LOQ), specificity and accuracy. Linearity was found to be in the range of 1000- 5000 ng/spot with significantly high value of correlation coefficient $r^2 = 0.990$. The LOD and LOQ were 582.2 and 192.14 ng/spot, respectively. As the method could effectively separate the drug from its degradation product, it can employed as a stability indicating one. The recoveries of cabergoline was 99.23%.

KEYWORDS

Bilayer tablet, L-Arginine, SSG, Poly vinyl pyrolidone, HPMC K100M, Light Magnesium Oxide

INTRODUCTION

Cabergoline an ergot derivative is a potent dopamine receptor agonist on D_2 receptors .It is used as first line of agent in treatment of prolactinomas due to higher affinity for D_2 receptors sites[1]. The secretion of prolactin by the anterior pituitary is mainly under hypothalamic inhibitory control, likely exerted through release of dopamine by tubero-infundibular neurons. Results of *in vitro* studies demonstrate that cabergoline exerts a direct inhibitory effect on the secretion of prolactin by rat pituitary lactotrophs. Cabergoline decreased serum prolactin levels in reserpinized rats[2].

***Address for Correspondence:**
Ms. Vanita M. Lasan
Assistant Professor, Department of Quality Assurance,
L.M. College of Pharmacy,
Ahmedabad- 380009, Gujarat, India.
E-Mail Id: vanita_lasan@yahoo.co.in

Receptor-binding studies indicate that cabergoline has low affinity for dopamine D_1, α1- and α2-adrenergic, and 5-HT_1 and 5-HT_2 serotonin receptors.

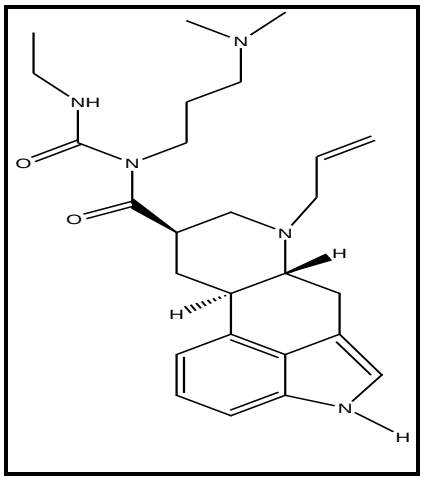

Figure 1: Structure of Cabergoline

Chemically it is 1-[(6-Allylergolin-8β-yl)-carbonyl]-1-[3-dimethlamino) propyle]-3-ethyleurea[3]. Its empirical molecular formula is $C_{26}H_{37}N_5O_2$. Chemical structure of cabergoline was Figure 1.

Following single oral doses of 0.5 mg to 1.5 mg given to 12 healthy adult volunteers, mean peak plasma levels of 30 to 70 pg/mL of Cabergoline were observed within 2 hours[4].

Various analytical methods has been reported for the determination of cabergoline. They are HPLC, RP-HPLC, UV visible spectroscopy, Mass spectroscopy, Differential scanning calorimetric (DSC)[5-13].

As on only few methods is available for their determination, however, it is essential to develop a suitable analytical method for the determination of cabergoline, because HPTLC methods have been widely used for routine quality control assessment of drugs, because of their accuracy, repeatability, selectivity, sensitivity and specificity. We have developed a simple, accurate method for cabergoline. Because analytical methods must be validated before use by the pharmaceutical industry, the proposed HPTLC method was validated in accordance with International conference on Harmonization (ICH).

MATERIAL AND METHODS

Chemicals and Reagents

Pharmaceutically pure samples of Cabergoline were obtained as a gift samples from Amneal pharma, Ahmedabad used as such without further purification. LR grade Methanol, Acetone, Hydrochloric acid, Sodium hydroxide, Triethylamine, 6 % Hydrogen peroxide purchased from Finar, Ahmedabad, India. LR grade Chloroform, Ammonia purchased from RFCL Ltd., New Delhi, India.

Instrumentation and Chromatographic Conditions

Analysis was performed with a camag HPTLC separation module equipped with camag cats4 software, Camag Linomat IV (Semiautomatic spotting device), and camag TLC scanner set at 280 nm. The combination of Chloroform: Methanol: Ammonia: (8:2:0.5 v/v/v) at room temperature with 15 min saturation time provided optimum polarity for proper migration, separation and resolution of cabergoline and its degradation products gives the R_f 0.65 ± 0.03. The U.V spectrum of cabergoline was taken at 280 nm wavelength using shimadzu UV 1800, with UV Probe software UV-Visible spectrophotometer (Shimadzu, Kyoto, Japan). All weighing were done on Shimadzu balance (Model AY 120).

Preparation of Standard Stock Solutions

Stock Solution of Cabergoline S1

Weighed accurately 10 mg of Cabergoline in to 10 mL volumetric flask and diluted with methanol up to the mark. (1000 µg/mL of cabergoline).

Working Standard Solution

Aliquots of S1 1, 2, 3, 4, 5 mL were transferred in series of 10mL of volumetric flasks and diluted up to the mark with methanol to prepare series of 100-500µg/mL concentrations.

Degradation Study

The drug content was employed for acidic, alkaline, and oxidant media and also for thermal and photolytic stress conditions. After the degradation treatments were completed, the stress content solutions were allowed concentration 10µl were spotted using Hamilton micro syringe into the TLC plate and the chromatograms were recorded to assess the stability of sample. Specific degradation conditions were described as follows.

Acid Degradation

Accurately weighed cabergoline (10 mg) was transferred to 50 mL round bottom flask and add 25 mL of 1.0 N HCl and was refluxed for 3 hours. Aliquot (1 mL) was pipetted out in 10 mL volumetric flask at 3 hour and was neutralized with 1.0 N NaOH and diluted with methanol up to the mark. The solution (10 µL) was spotted on TLC plate along with standard solution (Figure 2) and analysis was performed as described under chromatographic condition (Figure 3).

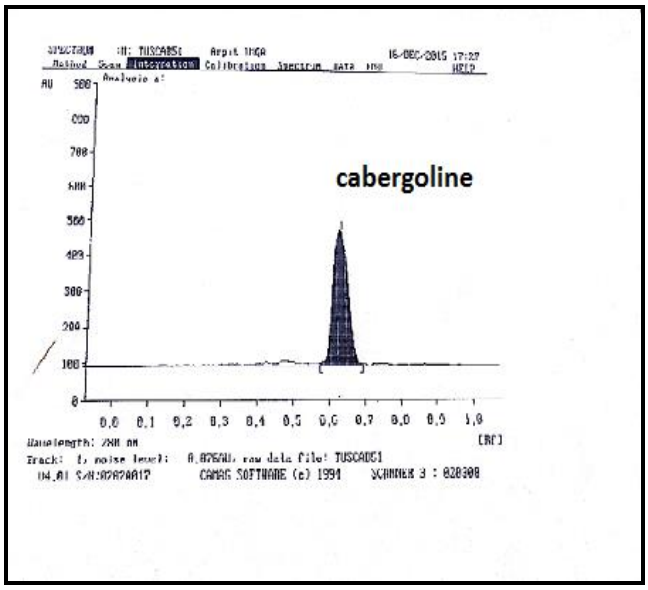

Figure 2: Standard chromatogram of cabergoline

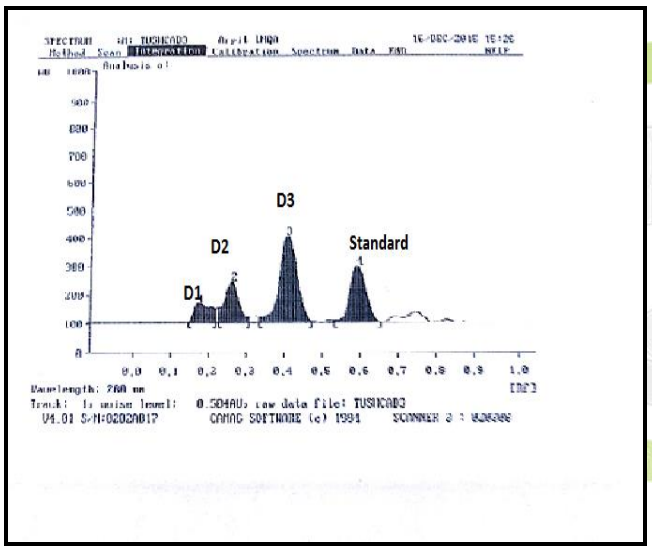

Figure 3: Chromatogram for Acid degradation of cabergoline

Base Degradation

Accurately weighed cabergoline (10 mg) was transferred to 50 mL round bottom flask and add 25 mL of 1.0 N Methanolic NaOH (2000 µg/mL) and was refluxed for 6 hours. Aliquot (1 mL) was pipetted out in 10 mL volumetric flask at 6 hour and was neutralized with 1.0 N HCl and diluted with methanol up to the mark. The solution (10 µL) was spotted on TLC plate along with standard solution and analysis was performed as described under chromatographic condition (Figure 4).

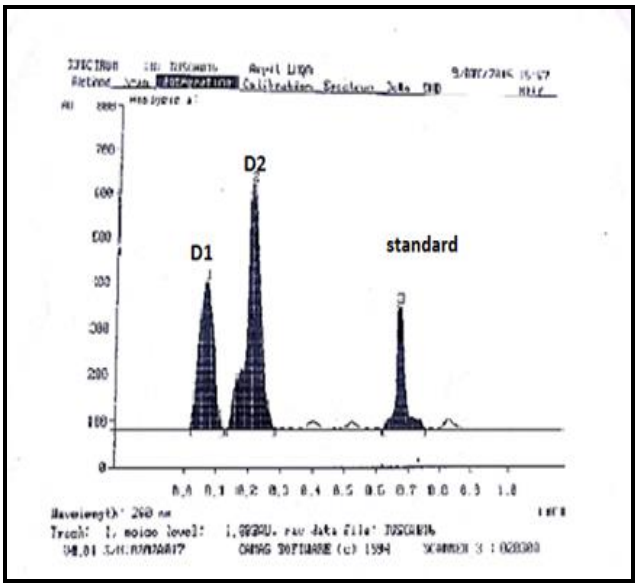

Figure 4: Chromatogram for Base degradation of cabergoline

Neutral Hydrolysis

Accurately weighed cabergoline (10 mg) was transferred to 50 mL round bottom flask and dissolved in methanol and add 25 mL of water (2000 µg/mL) and was refluxed for 24 hours. Aliquot (1 mL) was pipetted out in 10 mL volumetric flask at 24 hours and diluted with methanol up to the mark. The solution (10 µL) was spotted on TLC plate along with standard solution and analysis was performed as described under chromatographic condition (Figure 5).

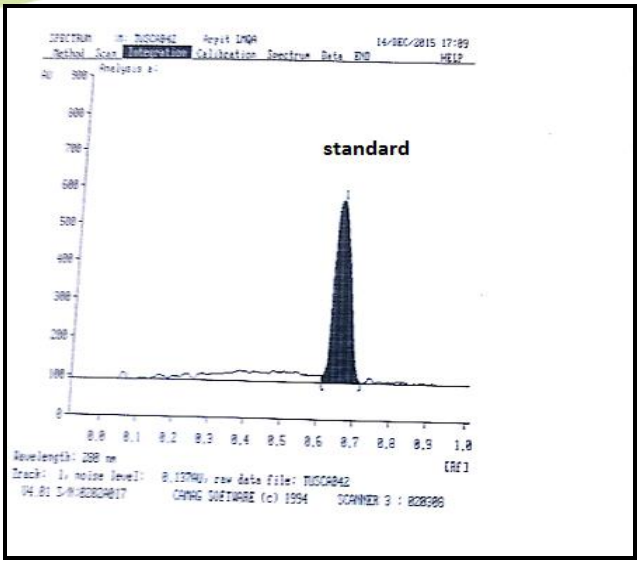

Figure 5: Chromatogram for Neutral degradation of cabergoline

Oxidation Degradation

Accurately weighed cabergoline (10 mg) was transferred to 10mL volumetric flask, hydrogen peroxide (5 mL, 6%) was added and diluted with methanol up to the mark and kept at room temperature. After 48 hours' time interval, aliquot (1 mL) was pipetted out in 10 mL volumetric flask and diluted with methanol up to the mark. The (10 µL) was spotted on prewashed TLC plate and analysis was performed as described under chromatographic condition (Figure 6).

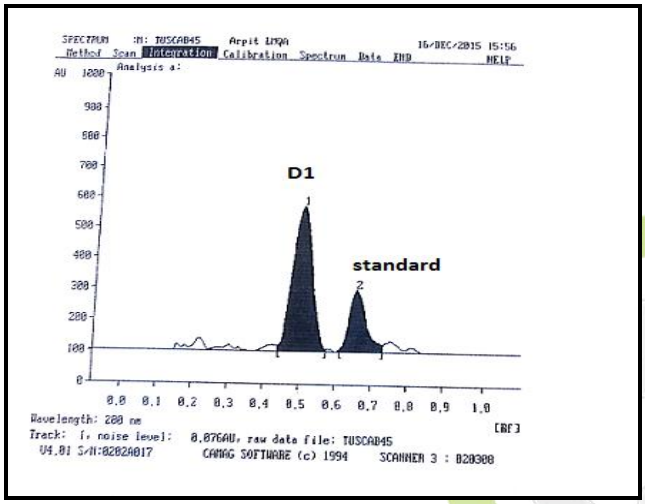

Figure 6: Chromatogram for Oxidative degradation of cabergoline

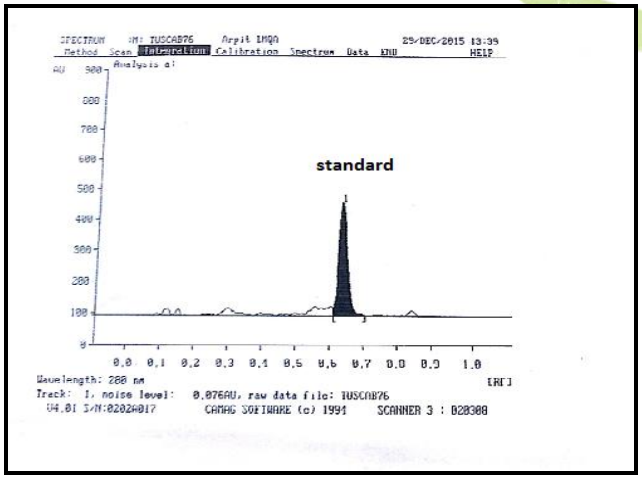

Figure 7: Chromatogram for Photolytic degradation of cabergoline

Photolytic Degradation

Accurately weighed cabergoline (10 mg) and transferred in porcelain dish having 25 mL methanol (2000 µg/mL) and was exposed to ultraviolet light for 6 hours. After 6 hours' time interval, aliquot (1 mL) was transferred to 10 mL volumetric flask and was diluted with methanol up to the mark. The solution (10 µL) was spotted on prewashed TLC plate and analysis was performed as described under chromatographic condition (Figure 7).

Dry Heat Induced Degradation

Accurately weighed cabergoline (10 mg) was taken in porcelain dish and exposed to a temperature of 80ºC for 24 hours in hot air oven. After 24 hours, 10 mg of the sample was accurately weighed and transferred to a 10 mL volumetric flask, dissolved in methanol and diluted up to the mark. Aliquot (1 mL) was transferred to a 10 mL volumetric flask and diluted with methanol up to the mark. The solution (10 µL) was spotted on prewashed TLC plate and analysis was performed as described under chromatographic condition (Figure 8).

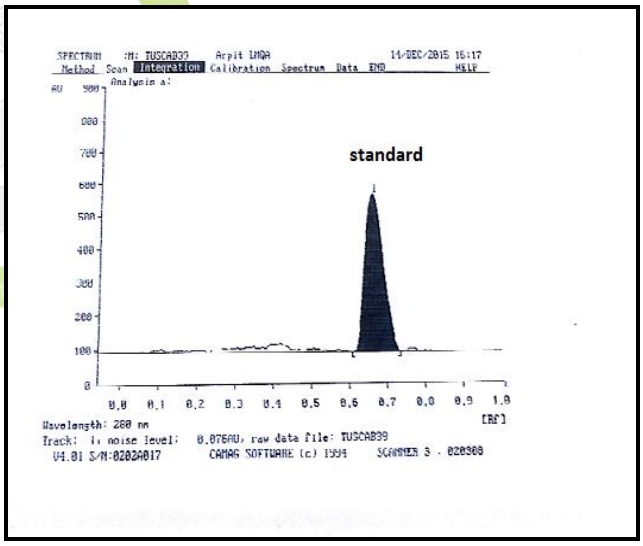

Figure 8: Chromatogram for Thermal degradation of cabergoline

RESULTS AND DISCUSSION

Method Development

Several tests were performed in order to get satisfactory separation-resolution of cabergoline in different mobile phases .The ideal mobile phase was found to be chloroform: methanol: Ammonia (80:20:5 v/v/v) and this mobile phase

used gave a very satisfactory and good resolution of cabergoline.

This work was focused on optimization of the conditions for the simple and rapid as well as low cost effective analysis including a selection of the proper mobile phase to obtain satisfactory results. The mobile phase conditions were optimized so there was no interference from solvent and excipients. Finalized chromatographic conditions were mentioned on below Table 1.

Table 1: Finalized chromatographic conditions

Stationary phase	Precoated Silica gel $G_{60}F_{254}$aluminium sheets 20 × 20 cm^2, layer thickness 0.2 mm.
Activation	TLC plates prewashed with methanol and activated in oven at 60°C for 5mins.
Mobile Phase	Chloroform : Methanol : Ammonia : (8 : 2 : 0.5 : v/v/v)
Chamber Saturation time	15 min
Temperature	Room Temperature
Migration distance	80 mm

Method Validation

Cabergoline in presence of its degradation product and impurities. A mixture of Chloroform: Methanol: Ammonia (8: 2 : 0.5 v/v/v) could provide sharp peaks of Cabergoline R_f = 0.65 ± 0.03 (Figure 9) and was found to be specific separation method, which can separate Cabergoline and its degradation product.

It was observed that activation of TLC plates (pre-washing with methanol followed by drying at 50º C for 5 min.) and pre-saturation of TLC chamber with mobile phase for 10 min ensures good reproducibility and peak shape. Photometric evaluation was performed at 280 nm, the absorbance maxima of Cabergoline absorbance/reflectance mode (Figure 10). Using optimized conditions, developed HPTLC method was validated in terms of linearity, limit of detection, limit of quantification, precision, and specificity.

Linearity

The linearity range for Cabergoline was found to be **1000 - 5000 µg/mL** (Table 2). Calibration curves were prepared and chromatogram is shown. (Figure 11) Regression for calibration curve of cabergoline was found to be **0.990**. The regression line equation is as follows:

y =7.413x ± 496.5 for Cabergoline

Where, y = Corresponding peak area

X = Concentration in ng

Table 2: Calibration curve data for cabergoline

Sr. No.	Conc^n (ng/spot)	Area (n=5) Mean ± SD	%RSD
1	100	1301.84 ± 11.65	0.89
2	200	1965.10 ± 41.21	2.09
3	300	2695.51 ± 36.61	1.35
4	400	3299.23 ± 49.48	1.49
5	500	4341.45 ± 56.65	1.30

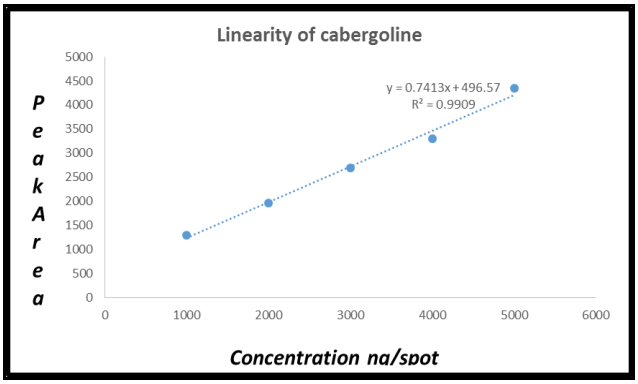

Figure 11: Calibration curve of cabergoline

Precision

Repeatability / Replication

An appropriate volume (10 µL) of Standard solution (3000 ng/spot) of was cabergoline spotted on precoated TLC plate. The plate was developed, dried and photo metrically analysed as described previously. Area and height of spot was measured six times without changing the position of plate. % RSD of obtained data was calculated. The data for repeatability scanner of peak area measurement is represented is based on six-time measurement of a same spot of cabergoline (3000 ng/spot). The % RSD for peak area and peak height were found to be 1.4 and 0.79 respectively (Table 3).The data for repeatability of sample application represented in (Table 4). The % RSD for peak area and peak height were found to be 0.82 % and 0.26% respectively.

Table 3: Repeatability of scanner peak area and peak height measurement

Sr. No.	Peak Area	Peak Height
1	2614.7	119.1
2	2628.5	120.0
3	2659.6	120.3
4	2675.2	121.6
5	2702.4	120.9
6	2700.6	121.5
Mean	2663.512	120.595
S.D	36.464	0.875
%RSD	1.4	0.79

Table 4: Repeatability of sample application

Sr. No.	Peak Area	Peak Height
1	2628.5	120.1
2	2628.5	120.9
3	2646.6	121.0
4	2654.3	120.6
5	2684.7	120.51
6	2648.6	120.6
Mean	2648.544	120.613
S.D.	18.91	0.29
%RSD	0.82	0.26

Reproducibility

Intermediate Precision

A. Intraday Precision

A variation of result within same day is called intraday variation. It was determined by taking 3 determinations of 3 concentrations from linear range. The data for intraday precision of method are summarized in (Table 5). The %RSD for intraday precision was found to be 0.76 % - 1.09 % for Cabergoline.

Table 5: Intraday precision

Sr. No.	(ng/spot) Concentration MEAN ± SD	%RSD
1	1000	1302.36 ±14.22	1.09106
2	2000	2684.33 ±15.94	0.5938
3	3000	4352.46 ±33.24	0.7637

B. Interday Precision

A variation of result amongst days is called interday variation. It was determined by taking 3 determinations of concentrations from linear range. The data for inter day precision of method are summarized in (Table 6). The %RSD for interday precision was found to be 0.89% - 1.86 % for Cabergoline.

Table 6: Interday precision

Sr. No.	Concentration (ng/spot)	Area (n=3) Mean± SD	%RSD
1	1000	1301.333±11.70	0.89
2	3000	2706.781±52.17	1.92
3	5000	4330.432±80.86	1.86

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated as (3.3×σ) /S and (10×σ) /S respectively; Where, σ is standard deviation of the Y intercept. S is slope of the calibration curve equation. LOD and LOQ were found to be 192.1 ng/spot and 582.2 ng/spot for Cabergoline.

Specificity

The purity of Cabergoline was ascertained by peak purity spectrum at three levels peak start, peak apex and peak end. It shows (Figure 12) correlation coefficient of 0.9996.

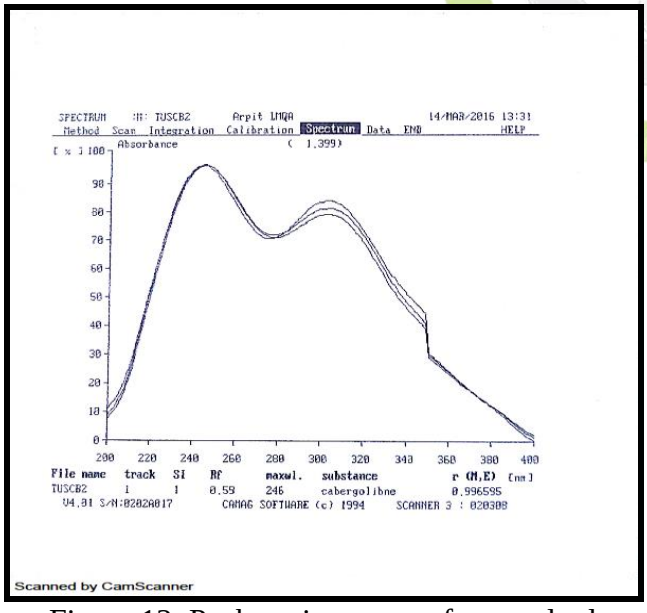

Figure 12: Peak purity spectra for standard cabergoline

Accuracy

Accuracy of an analysis is determined by calculating systemic error involved. It was determined by calculating recovery of the drug by standard addition method at three different concentration level of drugs. Along with standard calibration curve, assay formulation (5 µL) of solution containing cabergoline (200 µg/ml) was spotted on TLC plate under nitrogen atmosphere. 5 µL of standard solutions100 µg/ml, 200 µg/ml, 300 µg/ml were added on succeeding spots to obtain final concentration range of 300, 400, 500 ng/spot for cabergoline.

The plate was developed, dried and photo metrically analysed. The amount of drug was calculated by employing corresponding calibration curve equations. Average recovery obtained at all 3 levels was reported as % Recovery in Table 7.

Robustness

Robustness was performed to injected standard and sample solutions by small variation in the chromatographic conditions and found to be unaffected by small variations like ± 2% variation in volume of mobile phase composition with respect to methanol, ± 0.2 mm in detection wavelength,± 5 variation in run distance, ± 1 min variation saturation time. It was observed that there were no marked changes in the chromatograms, which demonstrated that the HPTLC method developed is robust. The % RSD was found as per in Table 8.

Assay of Tablet

To determine the content of cabergoline in conventional tablets (label claim: 2mg), twenty tablets were crushed, weight and equivalent weight were taken & dissolved in methanol. Then equivalent weight of the drug was transferred in 10 ml volumetric flask containing 5 ml methanol & diluted to 10 ml with methanol to obtain solution of cabergoline (200 µg/ml).Its % Assay was found 95.83 in Table 9.

Summary

A simple stability indicating HPTLC method has been developed and validated for estimation of cabergoline in presence of its degradation products and other impurities with linear range of 200-700 ng/spot and correlation coefficient 0.998.

Table 7: Recovery for Cabergoline

Level	Amount of cabergoline added (ng/spot)	Total amount	Total amount found (ng/spot)	Spiked amount of cabergoline recovered(ng/spot) mean ± SD (n=3)	% Recovery mean ± SD (n=3)
0	200	200	191.66±1.09	-	95.83±0.97
50	100	300	287.14±2.62	95.47±2.51	99.70±0.97
100	200	400	385.73±2.26	194.06±1.86	99.14±1.70
150	300	500	484.00±3.12	292.34±4.21	98.85±1.35
%Average Recovery ± SD =99.23±1.34					

Table 8: Robustness data for cabergoline

Parameters	Mean Area (n=3)			Standard Deviation (n=3)			%R.S.D. (n=3)		
	Normal Condition	Changed Condition		Normal Condition	Changed Condition		Normal Condition	Changed Condition	
		(-)	(+)		(-)	(+)		(-)	(+)
Mobile phase composition (± 0.2 ml)	2662	2337	2839	8.57	10.4	13.2	0.32	0.44	0.46
Detection wavelength (± 0.2 ml)	2662	2760	2872	8.57	10.5	6.65	0.32	0.38	0.23
Run distance (± 0.5 ml)	2662	2648	2573	8.57	12.5	11.1	0.32	0.47	0.43

Table 9: Assay results

Formulation	Amount claimed (mg)	Amount obtained (mg) (n=3) Mean ± SD	% Assay (n=3) mean ± SD
Tablet	2	191.66±1.09	95.83±0.97

Table 10: Summary for degradation profile of cabergoline

Sr. No.	Conditions	No. of Degradation Products	R_f of degradation products
1	Acid Hydrolysis Refluxed with 1 N HCl for 3 hours	2	0.34,0.45
2	Base Hydrolysis Refluxed with 0.5 N Methanolic NaOH for 2 hours	2	0.25,0.40
3	Neutral Hydrolysis Refluxed with water for 24 hours	No degradation	--
4	Oxidative Degradation R.T for 6 hours in 6 % H_2O_2	1	0.46
5	Photolytic Degradation 24 hours exposure to U.V rays	No degradation	--
6	Thermal Degradation 24 hours exposure to 80°C in Hot air oven	No degradation	--

Table 11: Summary for validation parameters of cabergoline

Sr. No.	Parameters	Results
1.	**Linearity range (ng/spot)**	1000-5000
2.	**Correlation Coefficient(r)**	0.9988
3.	**Precision** **1. Intermediate Precision (%RSD)** a. Intraday precision (n=3) b. Interday precision (n=3) **2. Repeatability (%RSD)** a. Repeatability of measurement of peak area (n=6) b. Repeatability of sample application (n=6)	 0.76-1.09 0.89-1.86 1.4 0.82
4.	**Limit of Detection (ng/spot)**	192.14
5.	**Limit of Detection (ng/spot)**	582.2
6.	**Accuracy (% Recovery)**	99.23±1.34
7.	**Specificity**	Specific

CONCLUSION

The validated RP-HPLC method employed here proved to be simple, fast, accurate, precise and robust, thus can be used for routine analysis of cabergoline.

AKNOWLEDGEMENT

The authors express their gratitude to Amneal Pharmaceuticals Ltd. (Ahmedabad, India) for the gift sample of cabergoline.

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