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Stability Indicating HPTLC Method for Determination of Moxonidine in Pharmaceutical Preparations

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Abstract: Stability indicating normal phase high performance thin layer chromatographic method was developed and validated for newer antihypertensive Moxonidine [MOQ] in tablet formulation. Developed method is simple, precise, quick and accurate for quantification of MOQ. MOQ and all its possible degradants are distinctly resolved on silica gel $60F_{254}$ HPTLC plates with mobile phase methanol, toluene and triethylamine in 4:6:0.1(v/v/v) parts. Densitometric quantification of moxonidine was performed at 266 nm. The band of MOQ at R_F value 0.59 ± 0.02 is markedly separated from bands of reported degradants. Linearity is studied by calibration plot of moxonidine from 400-1600 ng/band by height and area, with correlation coefficient of 0.997 and 0.997 respectively. Results of estimation of marketed formulation named *Moxovas* was found to be 100.22 ± 1.37 and 99.13 ± 1.09 by height and area respectively. Validation assures the method for being specific, accurate, precise and robust with prospective perspective capability of its application in routine analysis of MOQ in pharmaceutical preparations.
Key words: Moxonidine, Chromatography, Stability Indicating, Degradation, Validation, HPTLC.

INTRODUCTION

Moxonidine [MOQ] chemically, 4-chloro-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-6-methoxy-2-methyl pyrimidin-5-amine ($C_9H_{12}ClN_5O$), (Fig.1), is official in BP[1]. It is imidazolidine derivative approved as centrally acting antihypertensive, selective agonist at α_2-adrenoceptors and I_1-imidazoline receptor, this receptor subtype is found in medulla oblongata of brain. MOQ is white crystalline powder of molecular weight 241.7 which is sparingly soluble in methanol, slightly soluble in methylene chloride and very slightly soluble in water and acetonitrile. A widespread literature survey revealed few analytical methods for determination of MOQ. Reported methods for determination of MOQ are GC-MS [2], LC-MS [3] in plasma. However, no stability indicating HPTLC analytical methods for estimation of MOQ were found in literature yet.

Validation of developed analytical method ascertains that the method is quick, accurate, precise and robust and suitable for analytical estimation of MOQ in pharmaceutical preparations even in presence of degradants, excipients or impurities.

EXPERIMENTAL

Chemicals, Reagents and Solutions

Standard MOQ was obtained from Macleod Pharma Ltd., Mumbai, MS, India. *Moxovas* is the only marketed brand of MOQ in India, which is bought from local market. All solvents and reagents used were of AR grade, Whatmann filter paper Grade I, 0.45µm and double distilled water were used throughout the procedure.

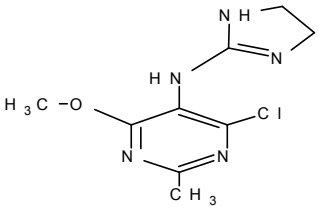

Fig. 1. Structural formula of Moxonidine

Preparation of Standard Solution

Fifty milligram of exactly weighed MOQ was dissolved in 25 ml methanol in 50 ml of volumetric flask. The volume was made up with methanol giving a standard stock solution of 1 mg/ml. Five parts of this standard stock solution was diluted up to 50 ml so as to get concentration of 100 µg/ml working standard.

Preparation of Sample Solution

Average weight of tablet was deduced by weighing twenty tablets. Triturated tablet powder equivalent to 5 mg of MOQ was transferred to 50 ml volumetric flask in sufficient methanol. Flask content was subjected to wrist shaker for 15 min and then sonicated for an hour to assure complete extraction and homogenization of API. Final volume was made up with methanol and resultant solution of 100 µg/ml was filtered.

Assay Procedure

Two bands of the working standard solution and six bands of the sample solutions of equal volume (10 µL) were spotted on the plate. The method applied is described in chromatographic conditions and instrumentation. The plate was developed and evaluated. The densitometric responses from the standard and sample were used to calculate the amounts of the drug in the tablet.

Force Degradation Study

Stress degradation is useful in establishing stability of drug, tracing degradation pathways and developing validated stability indicating analytical methods [4]. Stress studies as per ICH guidelines was performed by subjecting pure MOQ over wide range of pH, thermal, oxidative and photo conditions for different time intervals[5].

For hydrolysis study in extreme pH conditions, 50 mg MOQ was transferred in two flasks containing 25 ml methanol which is made up to 50 ml with 0.2M HCl and 0.2M NaOH respectively. Hydrolytic study under normal condition was performed by placing 50 mg MOQ in 50 ml of 50% Methanol (v/v). Half the content of these three round bottom flasks were kept on water-bath at 90°C under reflux and remaining half of each was placed at ambient temperature for 10 hrs.

Oxidative stress was studied by placing 25 mg of MOQ in 25 ml of 3% H_2O_2 in a flask at ambient temperature. Regular samplings from all the seven flasks were done at ½, 1st, 3rd, 5th, 8th and 10th hrs.

Photolytic and Thermal degradations were studied by evenly spreading 50 mg of pure MOQ in petri-dish and placing it covered under non-stray UV light and 100°C hot air oven respectively. Regular samplings were done on 1st, 3rd, 5th, 8th and 10th days and were analyzed for probable degradations.

The samples which showed no degradation at initial stress conditions were subjected to further severe stress conditions till a certain maximum limit had reached. Information on limit of maximum stress conditions were acquired from available regulatory guidelines, current pharmaceutical stress testing trends and practical limitations incurred due to physicochemical limitations of the molecule.

Force Degradation Study

Degradation samples, each of 5 ml from acidic, alkaline, neutral and oxidative degradation were withdrawn at defined time interval. The volume was made up to 10 ml with methanol. In case of photo and thermal degradation conditions 5 mg of pure MOQ from petri-dish were withdrawn at defined intervals and dissolved in 10 ml methanol, which were stored under refrigeration as stock solutions. All above stock solutions were further diluted with methanol to get 100 µg/ml concentrations, which were used for further chromatographic estimation.

Chromatographic Conditions and Instrumentation

Chromatographic method was developed on 10 x 10 cm aluminium HPTLC plates (Merck, Darmstadt, Germany) precoated with a 200 µm thick silica gel 60 F_{254} layer. All the plates were prewashed with methanol and activated at 115°C for 30 min before use. Ten micro liter samples were applied on 6 mm wide bands kept 3 mm apart using Hamilton syringe (Hamilton, Nevada, USA) and Camag Linomat IV automatic sample applicator (Muttenz, Switzerland) with an application rate of 7 s μL^{-1}. Conditioning of chamber and plate were achieved by saturating both with mobile phase vapors for 15 min respectively at room temperature (25± 2 °C). Densitometric scanning was performed with a CAMAG TLC scanner III installed with CATS 4 software (Version 1.4.1; CAMAG) in reflectance-absorbance mode at 266 nm. The slit dimensions were 5.00 mm × 0.45 mm with scanning speed of 20 mm/s. Deuterium lamp emitting UV radiation between 190 and 360 nm was acting as a source. Quantitation of chromatogram employs the basis of diffusely reflected light intensity determination.

HPTLC method development and optimization

Initially pure drug was run in methanol which showed movement of MOQ band beyond acceptable R_f range with slight irregular peak dimensions. Movement of MOQ in methanol is retarded by gradually increasing the proportion of less polar toluene with methanol from 1 part in ten up to 7.5 parts which provide satisfactory R_f value of 0.42 ± 0.02. Fineness in peak shape was brought with addition of 0.1 part of triethyl amine in mobile phase. When individual degradation samples were run in this mobile phase, degradants were noticed in acidic, alkaline, neutral and oxidative samples, deduced from reduction in peak height and area of MOQ. No degradations are found under thermal in 100 °C hot air oven and UV light placed for 10 days. Two out of four degradations i.e. neutral and oxidative are not visible on chromatogram perhaps due to their non-absorbance in UV range whereas, acidic and alkaline hydrolytic degradation bands were found overlapping in above developed mobile phase. Contrarily, reducing the concentration of toluene and increasing methanol proportionately had resulted in marked separation of acidic and alkaline degradation bands from that of MOQ. Thus ultimately optimized mobile phase comprising methanol: toluene: triethyl amine in 4.0:6.0:0.1 parts (v/v) which results into well resolved, symmetrical peaks with no tailing. *In-situ* UV spectrum of MOQ at 266 nm shows that no excepient interference was perceived in chromatogram. This wavelength seems to be appropriate for densitometric evaluation of all degradants with MOQ. A typical Densitogram of MOQ is depicted in **Fig. 2.**

Validation of Proposed Method

Validation of analytical methods is recommended to be performed during the phase of development to demonstrate that the method is suitable for its intended purpose [6]. Method is validated with respect to Linearity, LOD and LOQ, precision, accuracy and specificity.

Linearity

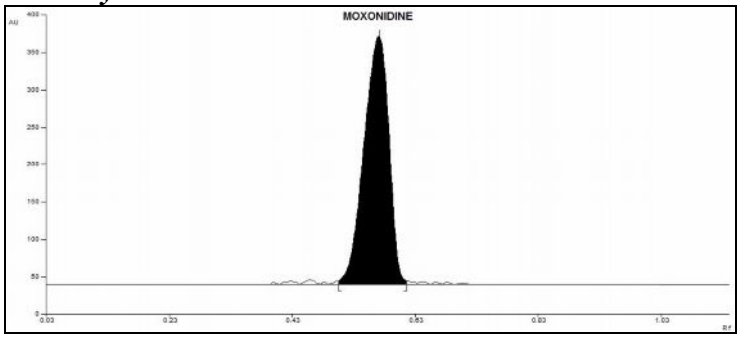

Fig. 2. HPTLC Chromatogram of Moxonidine.

The calibration curve was found to be linear over the concentration range 400-1600 ng/spot both by height and area with correlation coefficient of 0.998 and 0.998 respectively. Equations Y= 0.263x -11.03, RSD ± 2.60 and Y= 10.98x -2013.7, RSD ± 3.69 represents the calibration curve of MOQ by height and area respectively.

LOQ and LOD

Detection and quantitation limits were determined based on standard deviation of the response and the slope, where both values were deduced from the results of calibration curve [6]. LOD is the minimum quantity of analyte that can be detected in sample, but not necessarily quantitated, which was found to be 250.95 ng and 6.01 ng by height and area respectively. LOQ is the minimum level at which the analyte is quantifiable with requisite accuracy and precision, which was found to be 760.45 ng and 18.21 ng by height and area respectively.

Precision

System precision was performed by analyzing single standard six consecutive times. Assay method precision was done by carrying out six independent assays of a test sample of MOQ against a reference standard. The intermediate precision of the assay methods was evaluated from different analysts and different days investigation in the same laboratory to three samples (n = 3) in triplicate. Results of precision data is shown in **Table-I.**

Accuracy

Accuracy of the proposed method was established on the basis of recovery studies performed by standard addition method at three levels of labeled claim (i.e. 80,100 and 120 %) in duplicates. A known amount of pure drug was added to samples of tablet powder, which were then mixed, extracted and diluted appropriately for estimation. Accuracy data is summarized in **Table II.**

Table I. : System, method and intermediate precision data

Brand name	Parameter		System precision	Method precision	Intermediate precision		
					Interday	Intraday	Different analysts
MOXOVAS	Height	Mean ± SD	100.01 2.148	99.96 1.463	100.1 0.655	99.95 1.526	99.92 1.127
		% RSD	2.148	1.464	0.654	1.526	1.128
	Area	Mean ± SD	99.88 1.450	100.01 1.200	99.86 2.240	99.91 1.210	99.98 1.38
		% RSD	1.452	1.199	2.243	1.211	1.380

SD = Standard deviation, RSD = Relative standard deviation

Table II: Accuracy data

Moxovas Avg. Wt. 103.16 mg for 0.2 mg of Moxonidine						
Sr. No.	% Std addition	Wt. of tablet powder taken (mg)	*Moxovas*®			
			Wt. calculated (ng)		% Recovery	
			By Height	By Area	By Height	By Area
1)	80%	412.0	717.19	718.06	99.74	99.86
		413.0	720.80	719.50	99.48	99.3
2)	100%	412.0	789.82	778.91	99.34	97.97
		412.5	803.20	801.90	99.73	99.57
3)	120%	413.5	864.90	872.16	98.19	99.01
		413.0	870.85	874.60	99.48	99.91
			Mean		99.33	99.27
			± SD		0.57	0.72
			% RSD		0.57	0.72

SD = Standard deviation, RSD = Relative standard deviation

Table III: Specificity data

Moxovas®	Parameters		Normal	Acid	Alkali	Oxide	Heat	UV
% Labeled claim	*By Height*	Mean ± SD	98.90 ± 1.20	99.74 ± 0.61	99.85 ± 1.46	98.31 ± 1.44	99.43 ± 0.71	98.93 ± 0.68
	By Area	Mean ± SD	99.33 ± 1.09	99.16 ± 0.64	99.94 ± 0.90	98.16 ± 1.15	99.64 ± 0.49	99.43 ± 1.60

SD = Standard deviation

Specificity

The specificity studies were performed by attempting deliberate degradation of the tablet powder sample by subjecting under stress conditions of acidic (0.1 N HCl), alkaline (0.1 N NaOH), oxidative (3% H_2O_2) and sunlight at room temperature and thermal at 100°C each were kept for 24 Hrs. (Table III)

Robustness

Robustness of the analysis was validated by evaluating the influence of small but deliberate modifications in some experimental parameters on the R_f and peak shapes.

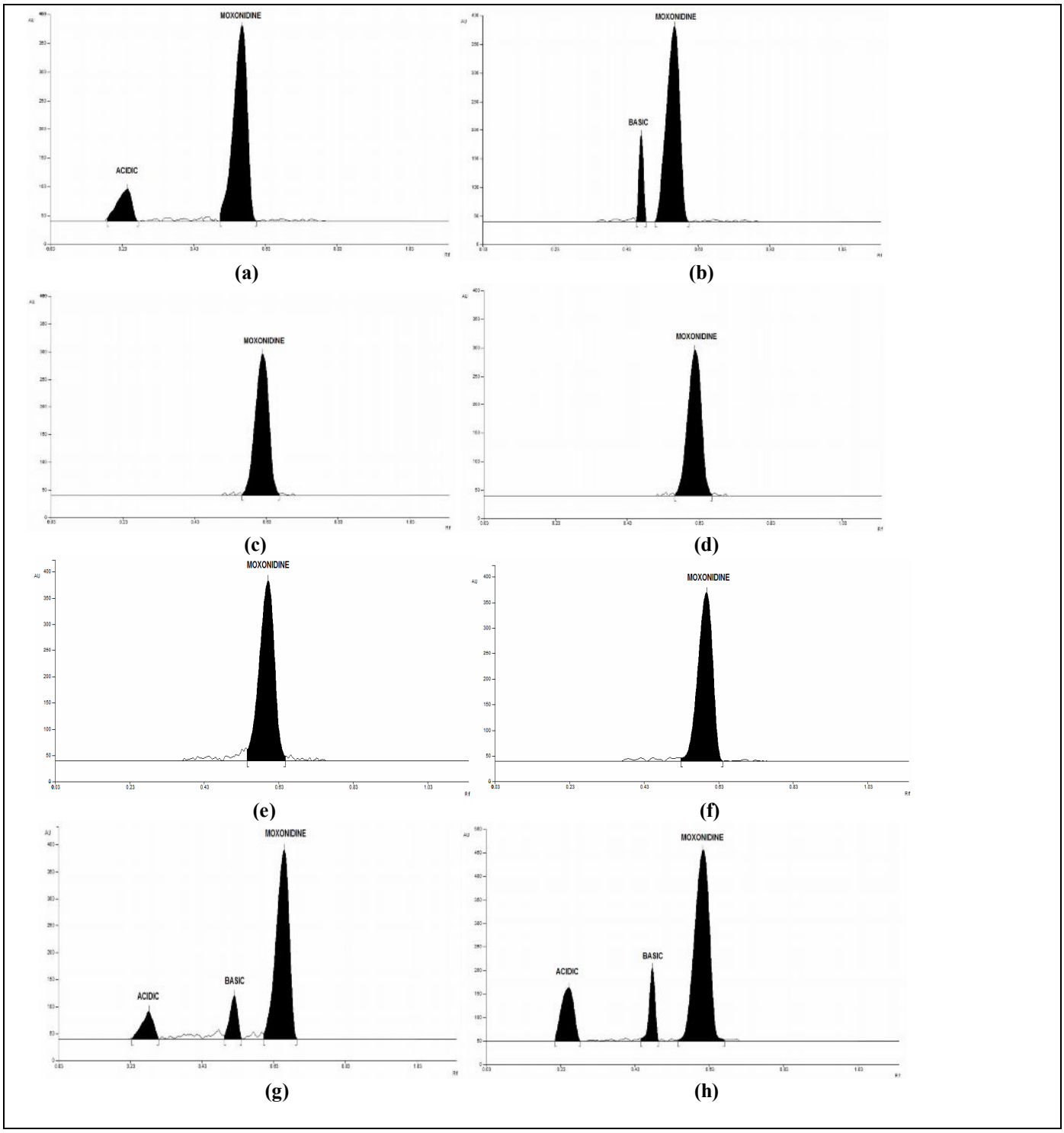

Fig. 3: Results of degradation studies in bulk drug: (a) 0.1M NaOH, 8 hr reflux at 90°C, (b) 1N HCl, half hr reflux at 90°C, (c) 3% H_2O_2, 10 hrs at RT (d) Neutral, 10 hr reflux at 90°C, (e) Under UV (254nm) at RT (f) Thermal, at 100°C, (g) Mixed 1M HCl and 0.1M NaOH of half and 8 hrs resp., (h) Mixed 1M HCl and 0.1M NaOH of 1 and 8 hrs resp.

RESULTS AND DISCUSSION

On the foresaid HPTLC plates, MOQ and its all probable degradants are distinctly baseline resolved by using methanol: toluene: triethyl amine in 4:6:0.1 parts as a mobile phase under defined conditions. Experimentally better quantitation is achieved at obtained R_f values of 0.59 ± 0.02, 0.24 ± 0.02 and 0.47 ± 0.02 for MOQ, acid and alkaline degradation products respectively. Furthermore, MOQ degradation in oxidative and neutral stress conditions were also assured from reduction in height and area of standard peak with due course of time. Nevertheless, oxidative and neutral stress degradants are invisible in chromatogram due to non-absorbance in UV-range. Improper mixing of mobile phase or saturation time results in unacceptable peak shape and R_f values.
Degradation was observed in alkaline condition on refluxing for 8 Hrs in 0.1M NaOH, whereas, no acidic degradation was found on refluxing for 10 Hrs in 0.1M HCl. On the basis of stability guidelines and degradation study trends acidic condition is exaggerated to 1M HCl. Degradants from 1M HCl stress sample was found in 30 minutes of reflux. In neutral and oxidative stress conditions degradation of MOQ was assured by reduction in height and area of it in samples of five and subsequent hours reflux. No degradation was perceived in acidic, alkaline and neutral samples placed at ambient temperature up to 10 Hrs. Results of degradation products under various conditions are shown in **Fig.3.**

CONCLUSION

The proposed method is thus ascertained for being quick, simple, precise and accurate for analytical purpose of MOQ in presence of its probable degradation product in bulk drug and pharmaceutical formulations. Validation of stability indicating analytical procedure covers the parameters of linearity, precision, accuracy, specificity and robustness which affirm the suitability of method for routine analysis in pharmaceutical formulations.

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