DEVELOPMENT OF STABILITY INDICATING CHROMATOGRAPHIC EVALUATION METHOD FOR MOXONIDINE IN PHARMACEUTICAL FORMULATIONS

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Objective

To develop Stability Indicating Analytical Method (SIAM) by HPTLC which is simple, specific , precise and accurate for estimation of Moxonidine in pharmaceutical preparations

Stability Indicating Analytical Method [SIAM] [1]

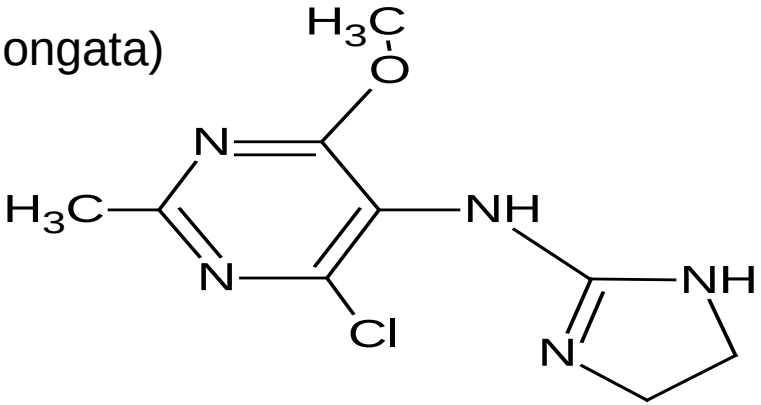
“Validated quantitative analytical methods that can detect the changes with time in the chemical, physical or microbiological properties of the drug substance or product and that are specific so that the contents of active ingredient, degradation products, and other components of interest can be accurately measured without interference”

Drug Profile

Chemical Category : Centrally acting Antihypertensive.

Mode of Action : Selective agonist at α_2-adrenoceptors and I_1-imidazoline receptor (this receptor subtype is found in medulla oblongata)

Structure :



Chemical Name : 4-chloro-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-6-methoxy-2-methylpyrimidin-5-amine[1]

Empirical Formula : $C_9H_{12}ClN_5O$

Molecular Weight : 241.7

Solubility : Methanol, Water, Acetonitrile

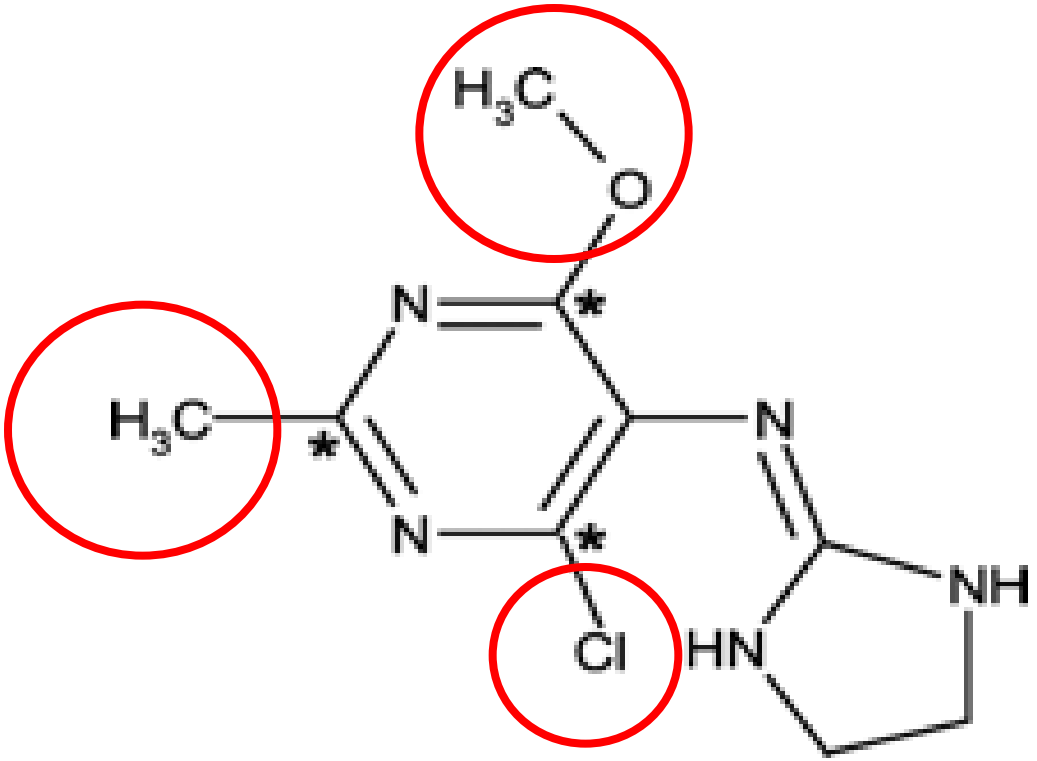
Ionization constant (pKa): **7.35 ± 0.03**

Half life : **2.86 ± 0.33 hrs**

T_{max} : **0.55 ± 0.17 hrs**

Bioavailability : **100.1 ± 9.9 %**

Chromophore groups



Methods Reported

*** Determination of Moxonidine in Human Plasma by LC-EI-MS[2]**

*** Determination of Moxonidine in Plasma by GC-MS[3]**

*** Determination of Moxonidine in Human Plasma by LC-MS and study of bioequivalence of moxonidine preparation[4]**

*** RP-HPLC Determination of Moxonidine HCl Tablets[5]**

Plan of Work

(a) Development of HPTLC Method for Estimation of Moxonidine in Pharmaceutical Preparations

- **Selection of suitable stationary phase and mobile phase**
- **Optimization of Chromatographic conditions**

  Study of linearity range
- **Estimation of drug in marketed formulation by proposed method**
- **Validation of the developed method as per ICH Guidelines**

(b) Forced Degradation Study of Moxonidine in Bulk Drug

- **Forced degradation products were prepared by subjecting it to various stress conditions so as to study the effects of wide range of pH, thermal, oxidative and light exposures**

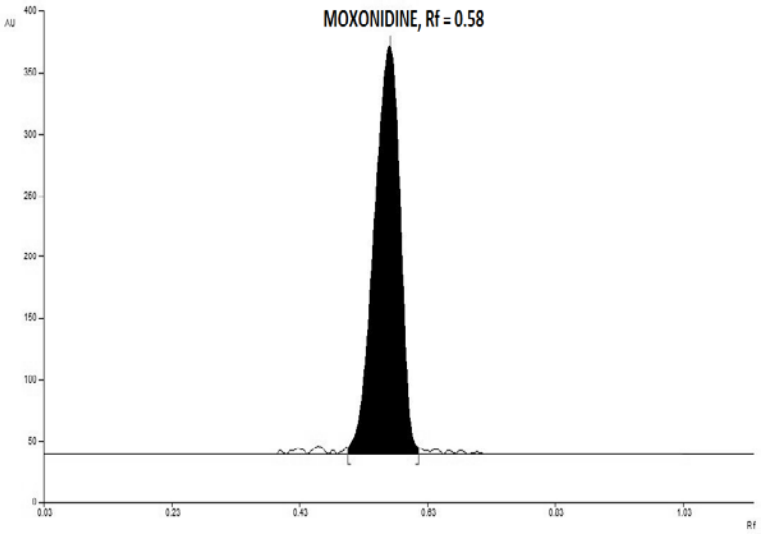
Chromatographic conditions

Stationary phase	**:**	**Precoated Silica Gel 60 F_{254} TLC Plate**
Mobile phase	**:**	**Methanol: Toluene:TEA, (4:6:0.1 v/v)**
Dimension	**:**	**10 x 10 cm**
Thickness	**:**	**200 μm**
Mode of application	**:**	**Band**
Band width	**:**	**6 mm**
Sample volume	**:**	**10 μl**
Application rate	**:**	**7 sec/μl**
Separation technique	**:**	**Ascending**
Development chamber	**:**	**Camag Twin trough glass chamber**
Saturation time	**:**	**10 min with mobile phase and spotted plate**
Migration distance	**:**	**80 mm**
Detection	**:**	**UV Densitometric scanning**
Scanning mode	**:**	**Absorbance/ Reflectance**
Scanning speed	**:**	**20 mm/sec**
Scanning wavelength	**:**	**266 nm**
Slit dimension	**:**	**5 × 0.45 mm**
Temperature	**:**	**25 ± 2 ^{0}C**

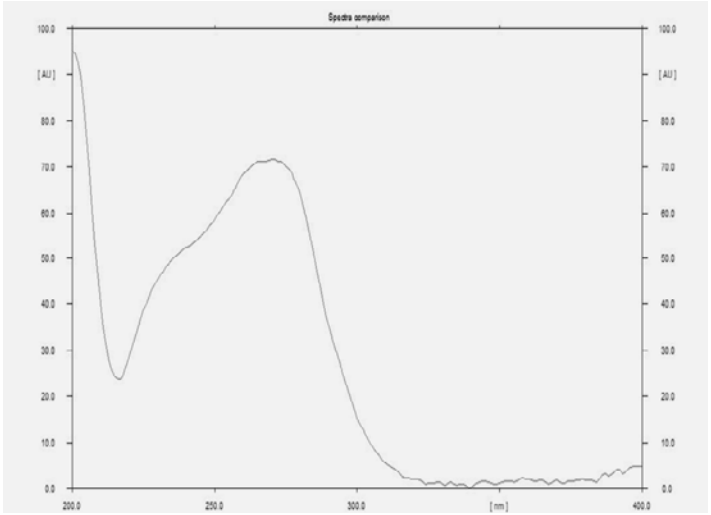
Experimental & Results

(a)Development of HPTLC Method for Estimation of Moxonidine in Pharmaceutical Preparation

Standard stock solution : 1mg/ml in methanol
Working standard : 100 µg/ml (diluted with methanol)



(A) Densitogram of standard drug



(B) Spectrum of Moxonidine

Estimation of Moxonidine in Tablet Formulations

Moxovas Avg. Wt. 103.16 mg for 0.2 mg of Moxonidine					
Sr. No.	**Wt. of tablet powder taken (mg)**	**Amt. Estimated in 5µl (ng)**		**% Drug Estimation**	
		By Height	**By Area**	**By Height**	**By Area**
1)	514.0	989.6	987.4	99.31	99.09
2)	518.0	1005.0	999.6	100.07	99.54
3)	518.0	995.5	968.9	99.90	97.23
4)	519.0	1011.0	1003.0	100.48	99.68
5)	516.0	988.5	988.1	98.81	98.77
6)	520.0	1036.0	1013.0	102.76	100.48
			MEAN	100.22	99.13
			± SD	1.3767	1.0990
			% RSD	1.3736	1.1086

Table 1: Results of Estimation of Moxonidine in Moxovas tablets

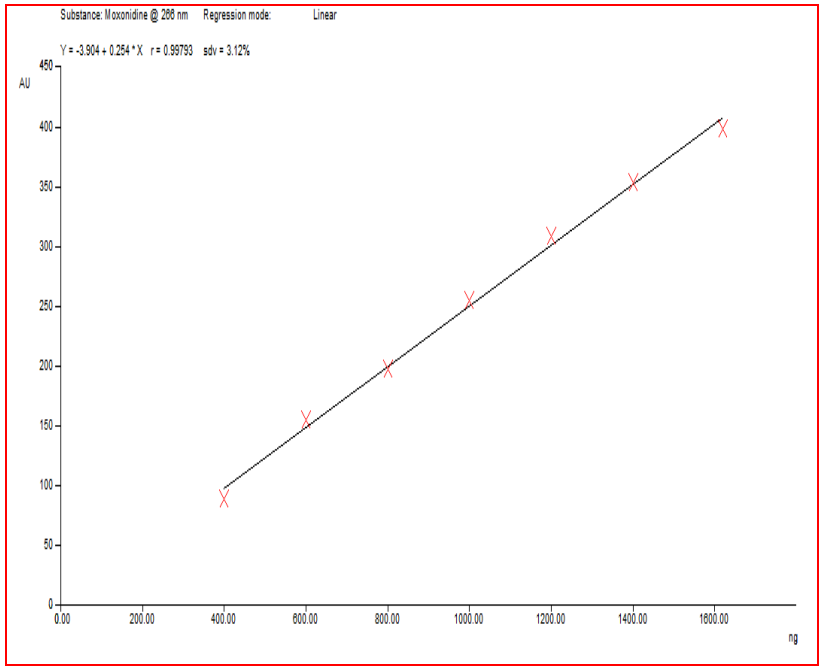
Validation of the developed method

Validation of developed method was carried out for linearity & range, LOD & LOQ, precision, accuracy, specificity, ruggedness and robustness.

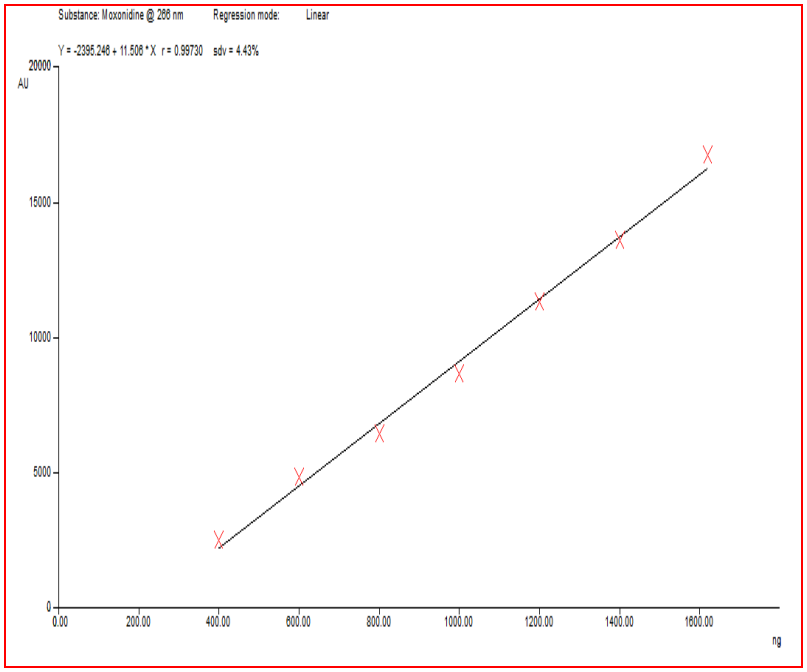
I. Linearity and Range:

Parameters	Values (at 266 nm)	
	By Height	By Area
Linear dynamic range (ng/spot)	400-1600	400-1600
Slope	0.254	11.50
Correlation coefficient (r)	0.997	0.997
LOD	250.95	6.01
LOQ	760.45	18.21

Study of linearity of response:



(A) Linearity by height



(B) Linearity by area

II. Precision

The results are summarized in Table 2.

	Parameters		System precision	Method precision	Intermediate precision		
					Interday	Intraday	Different Analyst
MOXOVAS®	Height	Mean ±SD	100.01	99.96	100.1	99.95	99.92
			2.15	1.46	0.6550	1.53	1.13
		% RSD	2.15	1.46	0.65	1.53	1.13
	Area	Mean ±SD	99.88	100.01	99.86	99.91	99.98
			1.45	1.20	2.24	1.21	1.39
		% RSD	1.46	1.20	2.25	1.21	1.39

Table 2 : Results of system, method and intermediate precision

III. Accuracy: The results are summarized in Table 3.

Moxovas Avg. Wt. 103.16 mg for 0.2 mg of Moxonidine						
Sr. No.	% Spiking level	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	0.5784	0.7220
				% RSD	0.5823	0.7273

Table 3 : Results of recovery study in Moxonidine Tablets

IV. Robustness:

The results are shown in Table 4.

Formulation	Parameter	Change in wavelength (±2nm)	
		264 nm	268 nm
MOXOVAS	By Height	100.90 ± 1.24	100.45 ± 1.33
	By Area	100.35 ± 1.44	99.87 ± 0.87

Table 4 : Results of Robustness

(b) Forced Degradation Study of Moxonidine

Stress Degradations were carried out under the following conditions considering ICH guidelines, physico-chemical properties of drug and current trends of study;

a) **Hydrolysis under acidic condition (1M HCl) at 90 ˚C & Room temp.**

b) **Hydrolysis under alkaline condition (0.1M NaOH) at 90 ˚C & Room temp.**

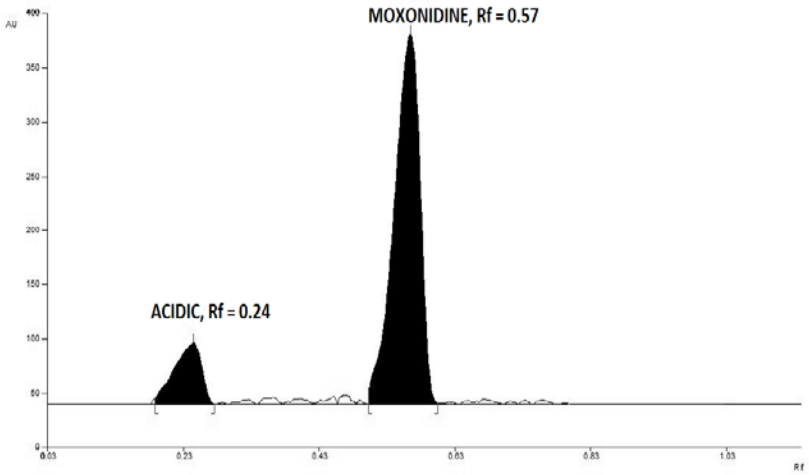
c) **Hydrolysis under Aqueous condition at 90 ˚C & Room temp.**

d) **Oxidation under the Peroxide solution (3% H2O2) at Room temp.**

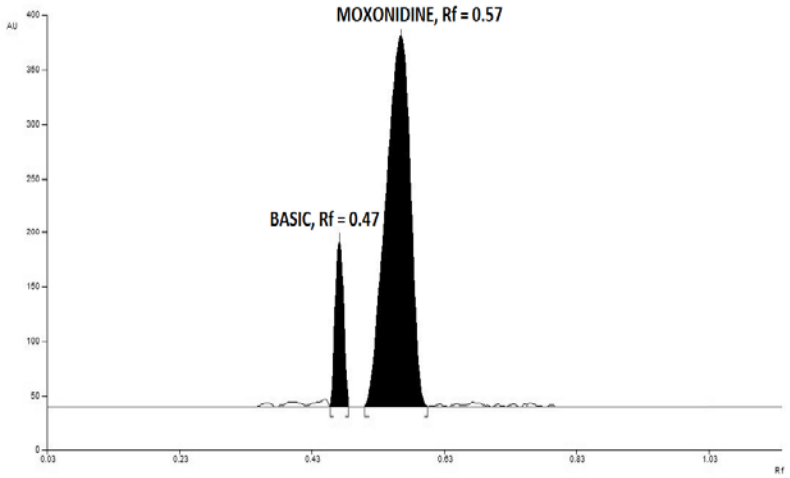
e) **Photodegradation under UV (254 nm) Lamp at Room temp.**

f) **Thermal degradation under 100 ˚C in Hot air oven**

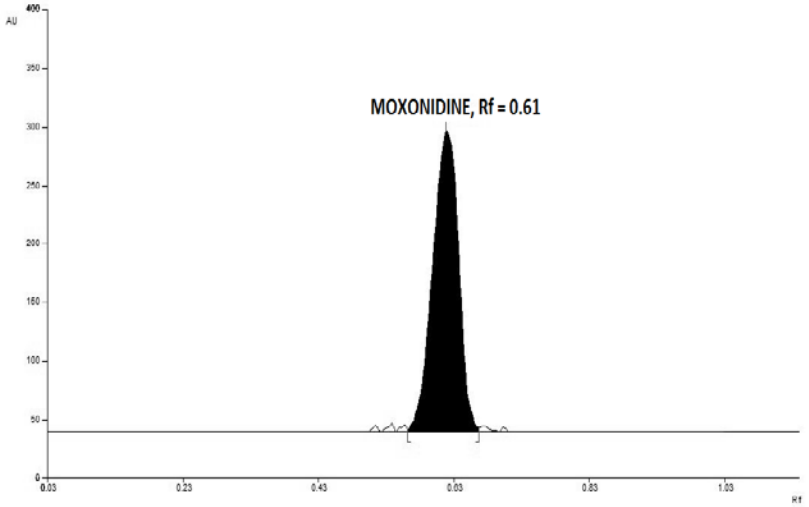
Forced Degradation Results



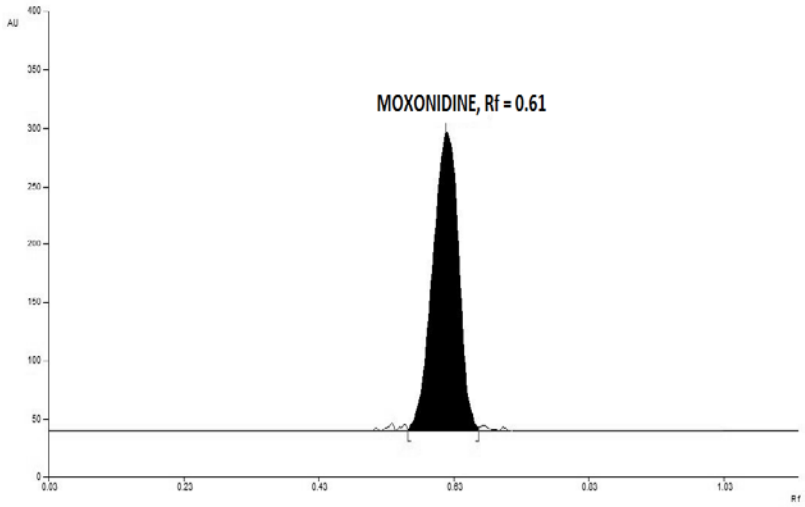
(a) Under 1M HCl Reflux, half hr.



(b) Under 0.1M NaOH Reflux, half hr.

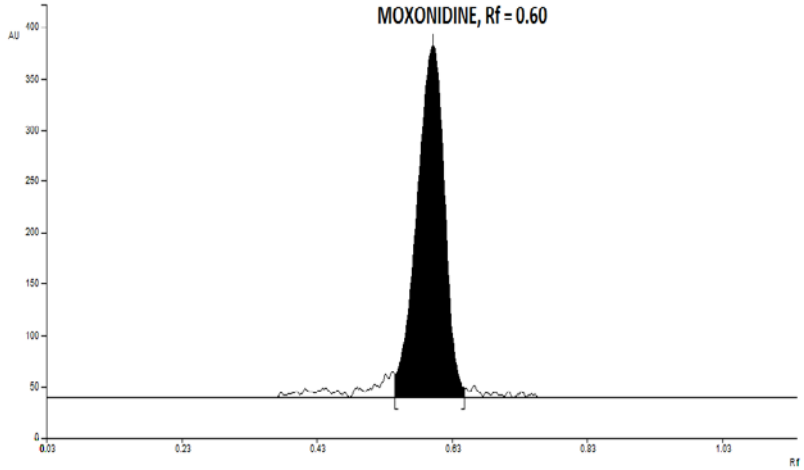


(c) Under 3% Peroxide at RT, 10 hr.

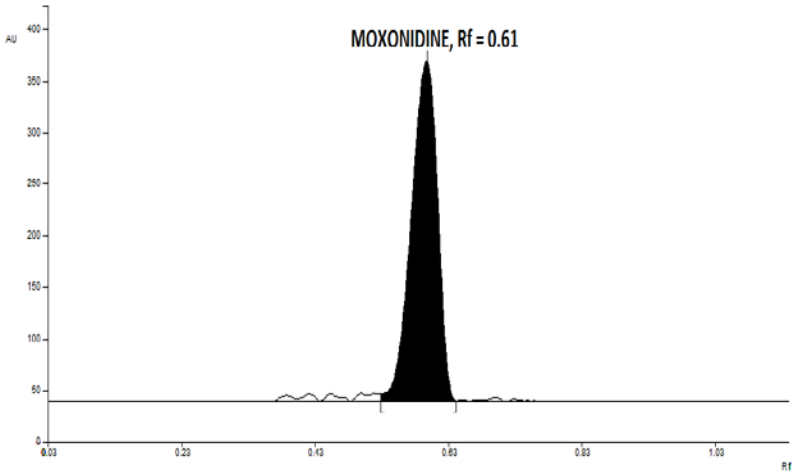


(d) Under Aqueous Reflux, 10 hr.

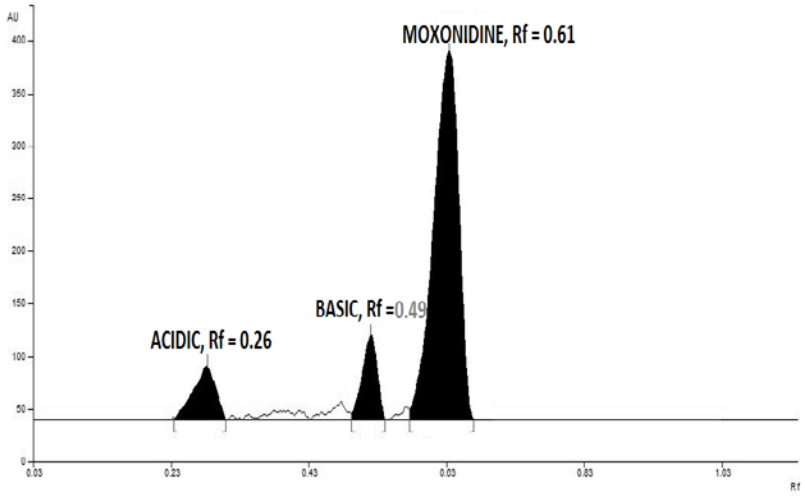
Forced Degradation Results



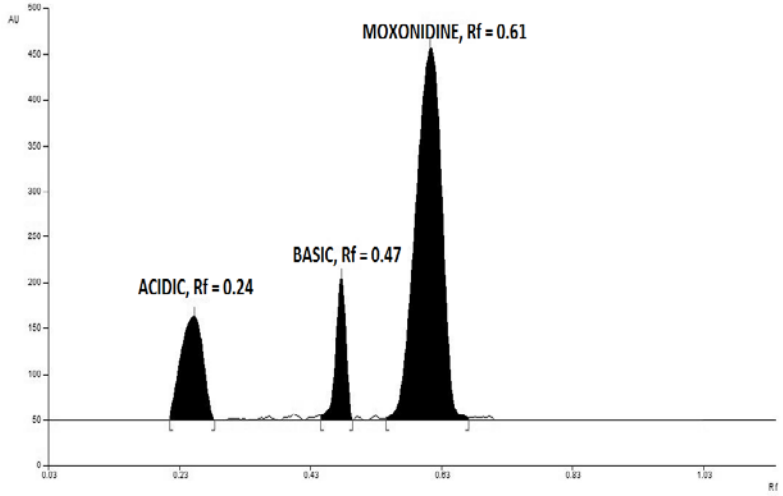
(e) Under UV Lamp at Room Temp.



(f) Under Thermal at 100 ˚C



(g) Mixed degradation of reflux hydrolysis, acidic & Basic of half & 8 hr. resp.



(h) Mixed degradation of reflux hydrolysis, acidic & Basic of one& 8 hr. resp.

Forced Degradation Results :

Parameters	Acid Reflux	Alkali Reflux	Neutral Reflux	Oxide Room Temp.	Thermal 100˚C	UV 254 nm
Mean ± SD	*86.27 ± 0.41*	*89.68 ± 1.05*	*79.84 ± 0.19*	*81.28 ± 0.37*	*99.54 ± 0.64*	*99.96 ± 0.34*

Table 5 : Results of forced degradation study

References

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2. **British Pharmacopoeia, 7th edn., British Pharmacopoeia Commission, London, 2009, pp. 4057**
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