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HPTLC validated stability indicating assay method for marketed herbal antihypertensive formulations

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ABSTRACT

Background: Reserpine is an indole alkaloid and found to be very unstable in various conditions previously. Here a simple, selective, precise and stability indicating high-performance thin-layer chromatographic method of analysis of reserpine was developed and validated.
Materials and methods: The method employed TLC plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consists of chloroform:acetone (7:3). This system was found to give well separated spots of reserpine at R_f (0.28 ± 0.02). The analysis of reserpine was carried out at 268 nm.
Results: The linear regression analysis data for the calibration plots showed good linear relationship with $r = 0.998$ and 0.999 with respect to peak height and peak area, respectively, in the concentration range 200–1000 ng per spot. The method was validated for accuracy, precision, recovery and robustness. The limits of detection and quantification were 109.85 and 292.10 ng per spot, respectively. The conditions prescribed in the parent drug stability testing guidelines (Q1AR) issued by International Conference on Harmonization (ICH).
Conclusion: The present study describes degradation of reserpine under different ICH prescribed stress conditions (acid and base hydrolysis, oxidation, dry and wet heat degradation and photo-degradation) and establishment of stability indicating HPTLC assay. The drug undergoes degradation under acidic and basic conditions, oxidation, dry and wet heat treatment and photo degraded. As the method could effectively separate the drug form its degradation products, it can be employed as a stability indicating one.

1. Introduction

Reserpine is an indole alkaloid chemically it is (3β, 16β, 17α, 18β, 20α) − 11, 17 − dimethoxy − 18 [(3, 4, 5 − trimethoxybenzoyl) − oxyl]yohimban − 16 − carboxylic acid methyl ester or 3, 4, 5 − trimethoxybenzoyl methyl reserpate (Fig. 1), used in lowering blood pressure, as tranquilizer etc.[1,2] Various methods are available for the analysis of reserpine in the literature like UV,[3,4] HPLC,[5] TLC,[6]colorimetric[7] IR, NMR and fluorescence,[4]but there is no reports on analytical methods for the estimation of degraded product of reserpine in bulk and its dosage form according to ICH guideline. Moreover, none of them is stability indicating method by HPTLC. The International Conference on Harmonization (ICH) guideline entitled 'stability testing of new drug substances and products' requires the stress testing to be carried out to explore intrinsic stability parameters of the active drug substances.[8] Stability indicating method can be defined that the method which determines the drug along with its degradation and reaction products. Acid, alkali, oxidation and light are very common stress conditions employed for stability studies using Titrimetric, spectrophotometric and chromatographic methods. Among chromatographic methods, nowadays, HPTLC is becoming a very common analytical technique where number of samples can be analyzed at a time for fast, reliable quantitative determination of drugs.[9–11] Hence in the present work it is decided to develop an accurate, specific, repeatable and stability indicating assay method for the determination of reserpine in the presence of its degradation products and related impurities as per ICH guidelines.

2. Experimental

2.1. Materials

Reserpine was purchased from Natural Remedies Bangalore, India. All chemicals and reagents of analytical grade were purchased from Merck Chemicals, India.

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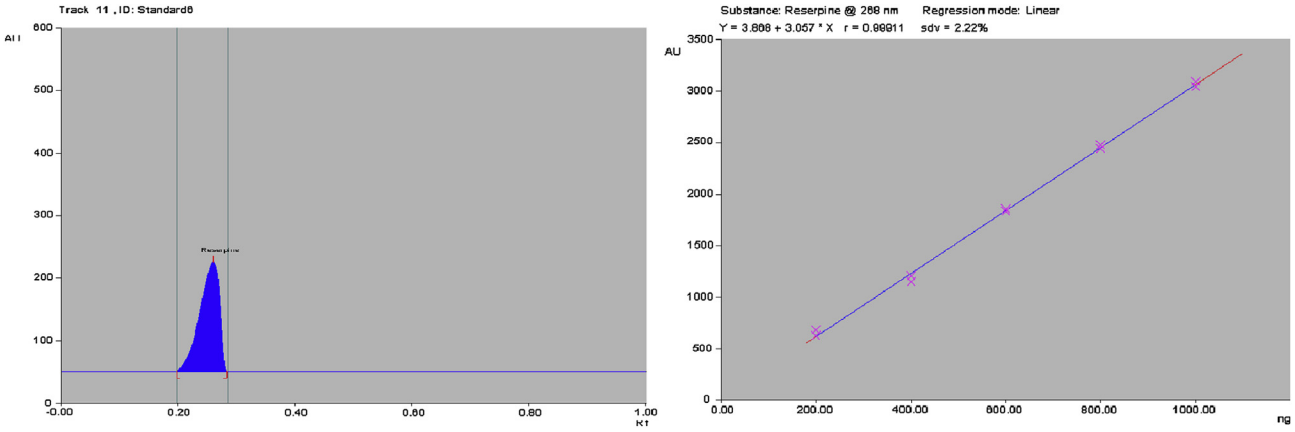

Fig. 1. Chromatogram of standard reserpine and its calibration curve.

2.2. HPTLC studies

Samples were applied using Camag Linomat-IV applicator using stationary phase of Silica gel plate 60F-254 (E. Merck, Germany) and mobile phase of chloroform:acetone (7:3 v/v). The bandwidth was 6 mm and length of chromatogram run was 90 mm. Densitometric scanning was performed on Camag TLC scanner at 268 nm.

2.3. Calibration curves of reserpine

A stock solution of reserpine (1 mg/ml) was prepared in methanol. Different volumes of stock solution 0.2, 0.4, 0.6, 0.8 and 1 ml were diluted to 10 ml with methanol to furnish solution containing 10, 20, 40, 60, 80, 100 μg/ml respectively. Each of these solutions (10 μL) spotted in duplicate on TLC plate to get concentrations of 100, 200, 400, 600, 800 and 1000 ng per spot of reserpine, respectively. The data of peak height/area versus drug concentration were analyzed by linear least-square regression.

2.3.1. Recovery studies

For recovery studies, samples were added with known amount of the standard reserpine and reanalyzed to determine percentage recovery.

2.3.2. Precision

Six replicates of the same spot measuring 1000 ng per spot of reserpine were applied and measured as a part of repeatability. The intra- and inter-day variation for the determination of reserpine was carried out at two different concentration levels of 200, 400 ng and 600 ng per spot.

2.3.3. Robustness of the method

With slight changes to mobile phase composition (chloroform and acetone mixture in proportion 6:4 and 7:3 v/v), temperature (±5%) and duration of saturation (±5%), robustness study was carried out in triplicate at 1000 ng/spot. Mean and RSD (%) of peak areas were calculated.

2.3.4. Limit of detection and limit of quantification

Limits of detection and quantification were calculated from the equation of graph of area, which is obtained by applying spots of concentrations in the lower part of the linear range of the calibration curve. Formulas used were detection limit = 3.3 * σ/s and quantification limit = 10*σ/s.

2.4. Analysis of the reserpine in marketed formulation

To determine the content of reserpine in marketed formulation A (labeled claim: Sarpagandha 62.5 mg) and formulation B (labeled claim: Sarpagandha 250 mg), the tablets were powdered and powder equivalent to 1 mg of reserpine was weighed. Dissolved in 100 ml distilled water. Filtered with Whatman filter paper. Take the residue in a separating funnel and powder with five 25 ml quantities of chloroform. Combine the chloroform layer. Evaporate the chloroform layer and dilute the residue with 10 ml methanol (100 μg/ml). The standard stock solution of formulation A and B was further diluted with the methanol to get the final concentration of about 10 μg/ml of reserpine. This final stock solution of formulation A and B are coded as solution B and C respectively.

2.5. Forced degradation of reserpine[12]

2.5.1. Acid and base induced degradation

In four different 5 ml reaction vials a volume of 1 ml of standard stock solutions A, B and C (0.5 mg/ml) were evaporated to dryness and a volume of 0.5 ml of 1 M hydrochloric acid was added to each vial. These vials are half inserted into a block heater at 9 0 °C for 10 min and cooled. A volume of 0.5 ml of Sod. Hydroxide (1 M) was added for neutralization. The contents are dissolved in methanol and diluted to a given claimed concentration of 10 μg/ml.

2.5.2. Preparation of base degradation products

In four different 5 ml reaction vials a volume of 1 ml of standard stock solutions A, B and C (0.5 mg/ml) were evaporated to dryness and a volume of 0.5 ml of 1 M Sod. Hydroxide was added to each vial. These vials are half inserted into a block heater at 90 °C for 10 min and cooled. A volume of 0.5 ml of 1 M hydrochloric acid was added for neutralization. The contents are dissolved in methanol and diluted to a given claimed concentration of 10 μg/ml.

2.5.3. Preparation of hydrogen peroxide induced degradation products

In four different 5 ml reaction vials a volume of 1 ml of standard stock solutions A, B, and C (0.5 mg/ml) were evaporated to dryness and a volume of 1 ml of 6% Hydrogen peroxide was added. These vials are half inserted into a block heater at 90 °C for 10 min and cooled. The contents are dissolved in methanol and diluted to a given claimed concentration of 10 μg/ml.

Table 1
Linear regression data for the calibration curves ($n = 5$).

Linear regression data for the calibration curves ($n = 5$)	
Linearity range (ng)	200–1000
Correlation coefficient	0.998
Slope ± S.D.	0.076 ± 2.53

2.5.4. Dry and wet heat degradation products

Two screw capped reaction vials were used. In the first vial put 1 mg of reserpine powder and in the second vial put 1 mg of reserpine with 10 μl water as a source of moisture. Similarly take standard stock solutions A, B and C in separate vials and evaporate to dryness. Both stored at 9 0° for 2 h in a hot air oven. The contents are dissolved in methanol and diluted to a given claimed concentration of 10 μg/ml.

2.5.5. Photochemical degradation products

The photochemical stability of the drug was also studied by exposing the 0.5 mg/ml of A, B, C and D solution to direct sunlight for three days (from 10.00 to 17.00 h on a wooden plank). The entire above degraded sample solutions were made up to a final concentration of 10 μg/ml methanol.

3. Results and discussion

3.1. Development of the optimum mobile phase

Different solvent compositions were tried to obtain well-defined spots. The mobile phase chloroform:acetone (7:3 v/v) and 10 min time of chamber saturation at room temperature gave good resolution with R_f value of (0.28 ± 0.02) for reserpine (Fig. 1).

3.2. Calibration curves

The linear regression data for the calibration curves ($n = 6$) as shown in Table 1 showed a good linear relationship over the concentration range 200–1000 ng per spot with respect to peak height and peak area (Fig. 1). No significant difference was observed in the slopes of standard curves.

3.3. Validation of the method

3.3.1. Recovery studies

The proposed method showed recovery of 99.58–99.75% for addition of 100 and 200 ng of drug respectively as listed in Table 2.

Table 2
Results of recovery studies ($n = 2$).

Theoretical content (ng)	Excess drug added to the analyte (ng)	Recovery (%)	% RSD
Sample (I)			
723.71	100	99.57	*0.018*
723.71	100	99.47	
723.71	200	99.61	
723.71	200	99.67	
		99.58	
Sample (II)			
662.49	100	99.75	*0.0044*
662.49	100	99.85	
662.49	200	99.63	
662.49	200	99.80	
		99.75	

Table 3
Results of Precision studies.

Amount ng/spot	% Estimation			
	Inter-day precision		Inter-day precision	
	Cardimap	Cardostab	Cardimap	Cardostab
200	98.74	99.05	96.20	96.52
400	99.82	99.58	99.31	98.79
600	99.74	99.85	99.51	99.40
Mean	99.43	99.49	98.34	98.23
S.D	0.18	0.08	1.72	1.15
% RSD	0.18	0.08	1.75	1.17

3.3.2. Precision

The repeatability study results were expressed in terms of %RSD and depicted in Table 3, which revealed intra- and inter-day variation of reserpine at two different concentration levels of 100 and 200 ng per spot.

3.3.3. Robustness of the method

Slight changes in mobile phase composition indicated robustness of the method as indicated by low values of % RSD (Table 4).

3.3.4. LOD and LOQ

Regression equation with a regression coefficient from calibration curve for peak area were obtained and results are depicted in Table 1. Detection limit and quantification limit was calculated by the method as described in Section 2.3.4 and found 200 and 1000 ng, respectively, and results are depicted in Table 5, which shows the sufficient sensitivity of the method.

3.3.5. Analysis of the prepared formulation

A single spot at R_f 0.28 was observed in the chromatogram of the reserpine samples extracted from tablets, which indicates no interference from the excipients. The reserpine content was found to be 97.13% & 82.47 respectively with a % RSD of 2.39% & 1.33 so it can be confirmed that reserpine is not degraded in the formulations. The low values of % RSD value shows suitability of this method for routine analysis of reserpine in pharmaceutical dosage forms.

3.3.6. Stability indicating property

Acid, base, hydrogen peroxide and light degraded samples showed well separated spots of pure reserpine from some additional peaks of degradation (Fig. 2). Table 6 is summarized with the

Table 4
Result of Robustness studies.

Parameters	% RSD		Mean % RSD
	Cardimap tab.	Cardostab tab.	
Mobile phase composition			
Chloroform:Acetone (6:4)	1.14	1.02	1.08
Chloroform:Acetone (7:3)	0.08	0.12	0.1
Temperature	0.75	0.18	0.46
	0.34	1.27	0.80
Duration of saturation	0.46	0.39	0.42

Table 5
Results of LOD & LOQ studies.

Drug	Equation of line	Limit of detection	Limit of Quantitation
Reserpine	By area $Y = 3.808 + 3.057 * x$	2.43 ng/spot	7.37 ng/spot
	By Height $Y = 10.874 + 0.077 * x$	109.85 ng/spot	292.10 ng/spot

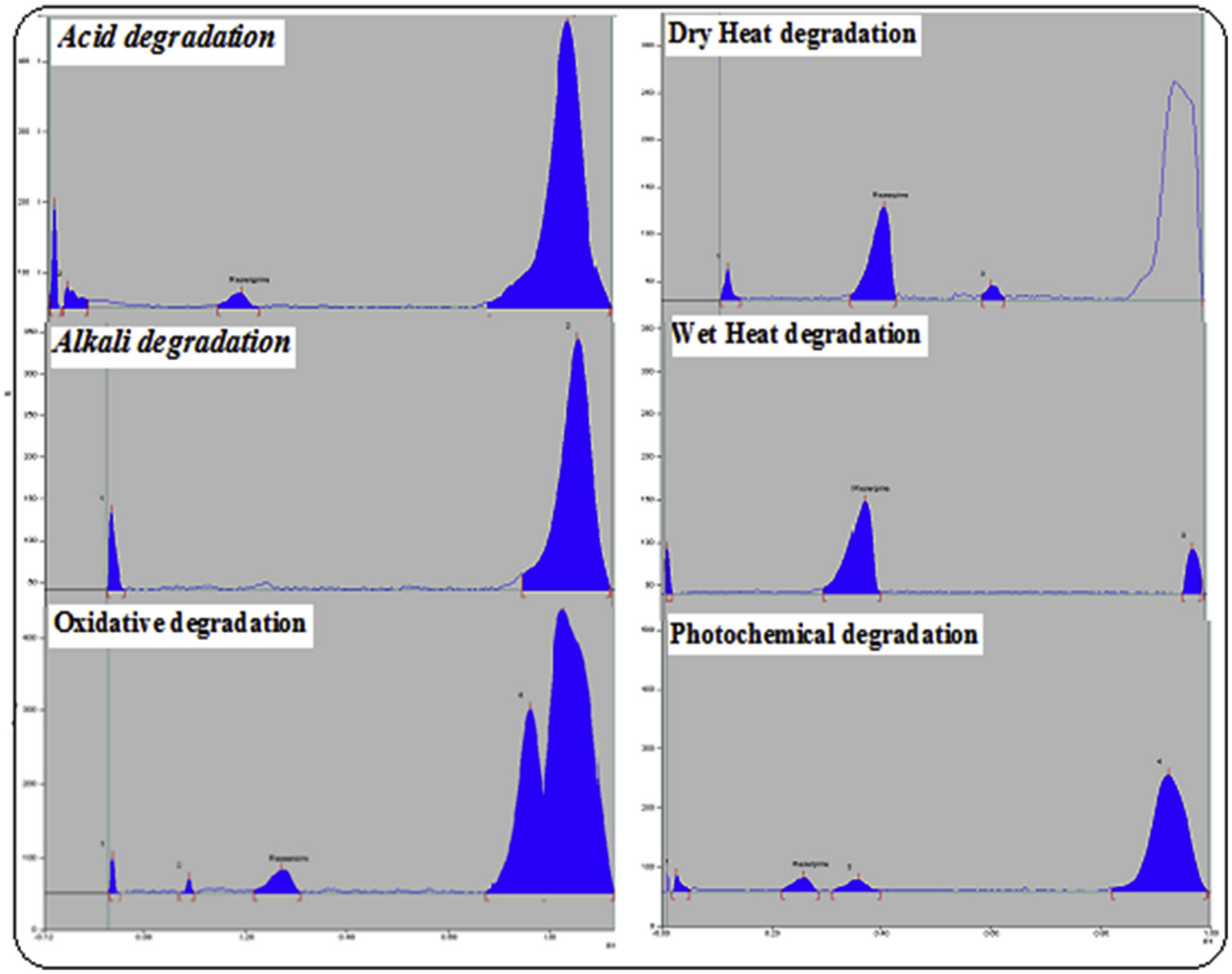

Fig. 2. Chromatogram of reserpine and its degradation product.

Table 6
Result of degradation study.

Sample	Condition	Time	% Recovery	Degradation peak R_f
Reserpine std.	1 N HCL at 90 °C	10 min	22.53%	0.04, 0.34
Cardimap	1 N HCL at 90 °C	10 min	27.25%	0.04, 0.35
Cardostab	1 N HCL at 90 °C	10 min	24.91%	0.05, 0.33
Reserpine std.	1 N NaOH at 90 °C	10 min	–	Nil
Cardimap	1 N NaOH at 90 °C	10 min	–	Nil
Cardostab	1 N NaOH at 90 °C	10 min	–	0.06
Reserpine std.	6% H_2O_2 at 90 °C	10 min	35.66%	0.16, 0.35, 0.83
Cardimap	6% H_2O_2 at 90 °C	10 min	37.30%	0.34, 0.40, 0.60, 0.84
Cardostab	6% H_2O_2 at 90 °C	10 min	19.38%	0.24, 0.33, 0.84
Reserpine std.	Hot air oven 90 °C (dry heat)	2 h	36.93%	0.34, 0.56
Cardimap	Hot air oven 90 °C (dry heat)	2 h	27.25%	0.03, 0.35
Cardostab	Hot air oven 90 °C (dry heat)	2 h	24.91%	0.05, 0.34
Reserpine std.	Hot air oven 90 °C (Wet heat)	2 h	36.12%	0.35, 0.79
Cardimap	Hot air oven 90 °C (Wet heat)	2 h	27.41%	0.04, 0.35
Cardostab	Hot air oven 90 °C (Wet heat)	2 h	25.80%	0.03, 0.34
Reserpine std.	Expose to sunlight	3 days	23.01%	0.28, 0.33
Cardimap	Expose to sunlight	3 days	6.5%	0.31, 0.32
Cardostab	Expose to sunlight	3 days	15.02%	0.34

details of degradation products with their R_f values, content of reserpine remained, and percentage recovery calculated.

4. Conclusion

The present paper confirms the development of validated, precise, specific, accurate and stability indicating HPTLC technique for determination of reserpine as bulk drug and in pharmaceutical formulations. Repeatability and selectivity of the method is proven by statistical analysis. The present method is stability indicating one because well separated reserpine from its degradation product can be quantified easily. Study of degradation products i.e. impurity profile of reserpine can be future scope of the present research work.

Conflicts of interest

All authors have none to declare.

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