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STABILITY INDICATING ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF RESERPINE AND DIHYDRALAZINE IN PURE AND MARKETED DOSAGE FORM BY USING HPLC

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ABSTRACT

A rapid and precise Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method has been developed for the validation of Reserpine (RES) and Dihydralazine (DHZ) in its pure form as well as in tablet dosage form. Chromatography was carried out on an Altima C18 (4.6 X 150 mm; 5µm) column using a mixture of ACN, Methanol and Phosphate buffer pH 4.6 (10:25:65 v/v) as the mobile phase at a flow rate of 1.0 ml/min, the detection was carried out at 215 nm. The retention time of Dihydralazine and Reserpine was 2.344 and 3.286 ±0.02 min respectively. The method produced linear responses in the concentration range of 10-50 mg/ml of Dihydralazine and 2.5-12.5 mg/ml of reserpine. The method precision for the determination of assay was below 2.0 % RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

KEYWORDS: RP-HPLC, ACN (Acetonitrile), Reserpine, Dihydralazine.

INTRODUCTION

Reserpine, an adrenergic antagonist, and Dihydralazine, a vasodilator, both are potential antihypertensive medications. In addition, reserpine also acts as antipsychotic.

Hydralazine apparently lowers blood pressure by exerting a peripheral vasodilating effect through a direct relaxation of vascular smooth muscle. The preferential dilatation of arterioles, as compared to veins, minimizes postural hypotension and promotes the increase in cardiac output. Hydralazine usually increases renin activity in plasma, presumably as a result of increased secretion of renin by the renal juxtaglomerular cells in response to reflex sympathetic discharge. This increase in renin activity leads to the production of angiotensin II, which then causes stimulation of aldosterone and consequent sodium reabsorption. Tolerance to the antihypertensive effect of the drug develops during prolonged therapy, especially if a diuretic is not administered concurrently.[1,4]

Reserpine acts through inhibition of the ATP/Mg^{2+} pump responsible for the sequestering of neurotransmitters into storage vesicles located in the presynaptic neuron. The neurotransmitters that are not sequestered in the storage vesicle are readily metabolized by monoamine oxidase (MAO) causing a reduction in catecholamines.[1,3,5]

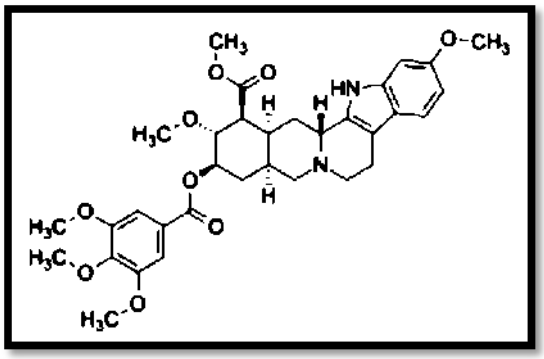

Figure 1: Structure of Reserpine.

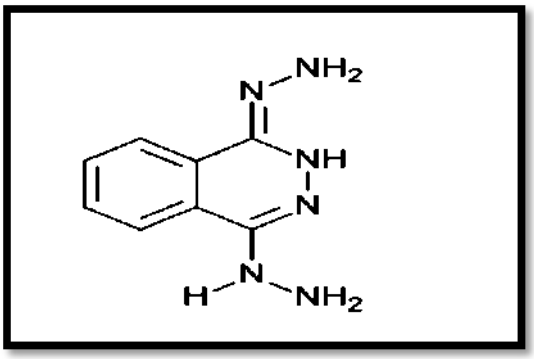

Figure 2: Structure of Dihydralazine.

High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantify the compounds that are present in any sample that can be dissolved in a liquid. Today, compounds in trace concentrations as low as parts per trillion (ppt) may easily be identified. HPLC can be, and has been, applied to just about any sample, such as pharmaceuticals, food, neutraceuticals, cosmetics, environmental matrices, forensic samples, and industrial chemicals. Nowadays, High Performance Liquid Chromatography (HPLC) has become one of the most important and common analytical techniques due to its high reliability and accuracy.[2,7]

MATERIALS AND METHODS

Chemicals

Analytically pure samples of Hydralazine and Reserpine were procured as gift samples from Hetero Labs Pvt. Ltd. (Hyderabad). Water, Methanol and Acetonitrile (HPLC Grade) were obtained from Merck Specialities Pvt. Ltd. (Mumbai).

Instrumentation and Chromatography

HPLC method development and validation was done using WATERS HPLC with auto-sampler, Software – EMPOWER-2, Stationary phase - C18 columns like ODS and Zodiac columns Altima C18 (4.6×150mm, 5µ); Alliance 2695 separation module and 996 PDA Detector.

Mobile Phase

Phosphate buffer (pH 4.6), Methanol and Acetonitrile were mixed in the proportion 65:25:10.

Standard Solution

Standard stock solutions (1 mg/ml) of drugs were prepared separately dissolving 10 mg of each drug separately in 10 ml of the mobile phase. From the standard stock solutions, mixed standard solutions were prepared to contain 100 µg/ml.[11,12]

Preparation of Sample Solution

Weigh and powder around 10 mg equivalent weight each of Dihydralazine and Reserpine sample into a 10 mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make the volume up to the mark with the same solvent. Further pipette out 0.75 mL of above stock solution into a 10ml volumetric flask (it contains 0.3 ml of Dihydralazine and 0.75 ml of Reserpine) and dilute up to the mark with diluent.[11,12]

Validation of HPLC method

The work validated for the parameters like linearity, precision, accuracy, Limit of Detection (LOD) and Limit of Quantification (LOQ).

For the linearity studies, calibration curves of Reserpine and Hydralazine standard solutions were constructed with concentrations ranging from 2-16 µg/ml each and correlation coefficients were determined.

To know the accuracy of the method, recovery studies were done by addition of standard drug solution to sample at three different levels 50%, 100%, 150% of the test concentration and the contents were re-analysed by the proposed method.

Precision of the method was checked in respect to repeatability and ruggedness. The % RSD was determined by six replicate injections of same standard solution on the same day under same experimental conditions. Ruggedness was assessed by injecting the same standard on different days. % RSD was determined in order to assess the ruggedness of the method.

Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined by injecting progressively low concentrations of the standard solutions.

RESULTS AND DISCUSSION

Method Development

The solutions of Reserpine and Hydralazine were injected into the HPLC system and run in different solvent systems as mobile phases. Finally, Buffer pH 4.6: Methanol: Acetonitrile (65:25:10v/v) was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for both Reserpine and Hydralazine. Representative chromatogram of mixed standard of Reserpine and Hydralazine is shown in Figure 3.

From the standard stock solution further dilutions were done using mobile phase and scanned over the range of 200-400 nm and the spectra were overlaid. It was observed that at 215 nm both Reserpine and Hydralazine showed considerable absorbance and therefore selected as detection wavelength.

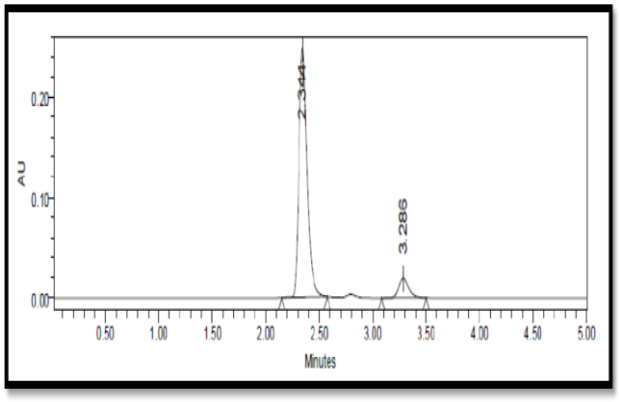

Figure 3: Chromatogram o Standard solution of RES and DHZ.

Method validation

The method was validated as per ICH guidelines.

Linearity

The linear relationship was observed between the peak area and concentration over the range of 2-16 µg/ml for

both the drugs. The method was proven to be linear in the above range as the correlation coefficient was 0.998 for both the drugs. Correlation coefficient, y-intercept, slope of regression line are shown in the Figure 4 and 5.

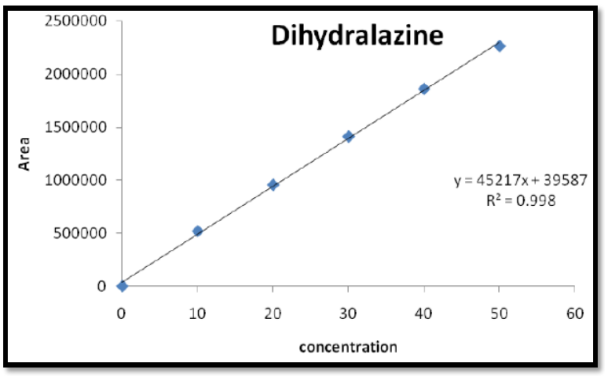

Figure 4: Calibration graph for Dihydralazine.

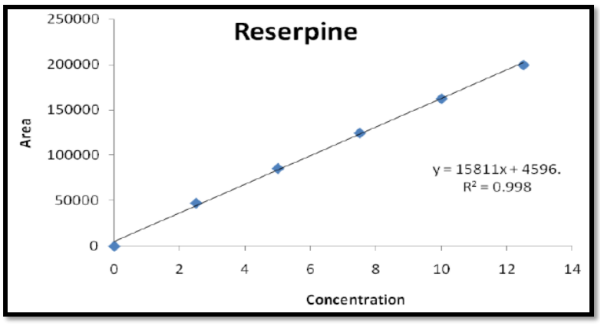

Figure 5: Calibration graph for Reserpine.

Accuracy

Accuracy is generally assessed by analyzing the samples with the known concentration and comparing the measured value with the true value. The measured values were obtained by conducting recovery studies. % Recoveries are shown in Table 1.

Table 1: % Recovery of RES and DHZ.

Drugs	% Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery
Reserpine	50%	63467	3.75	3.72	100.8
	100%	124353.3	7.5	7.57	100. 9
	150%	178607.7	11.25	11.20	99.5
Dihydralazine	50%	716072.7	15	14. 9	99.3
	100%	1404258	30	30.1	100.3
	150%	2064609	45	44.7	99.3

Repeatability

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions.

Recorded the peak areas and calculated % RSD. The %RSD values of RES and DHZ are shown in Table 2.

Table 2: %RSD values of RES and DHZ.

Drug	% RSD
Reserpine	0.5
Dihydralazine	0.22

Robustness of RES and DHZ

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1 ml/min and mobile phase ratio variation was observed from more organic phase to less organic phase ratio for Dihydralazine and Reserpine. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Dihydralazine and Reserpine were injected by changing the conditions of chromatography.

There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count. The results for robustness are given in Table 3.

Table 3: Results for Robustness of RES and DHZ.

Drugs	Parameter used for sample Analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Dihydralazine	Actual Flow rate of 1.0 mL/min	1308495	2.344	5568.2	1.3
	Less Flow rate of 0.9 mL/min	1300148	2. 244	5922.2	1.2
	More Flow rate of 1.1 mL/min	1306476	2.243	5868.8	1.2
	Less organic phase	1304520	2.345	5836.2	1.2
	More organic phase	1207845	2.344	5282.6	1.1
Reserpine	Actual Flow rate of 1.0 mL/min	124505	3.286	6098.1	1.2
	Less Flow rate of 0.9 mL/min	156550	3.181	5999.1	1.2
	More Flow rate of 1.1 mL/min	122702	3.181	5989.2	1.1
	Less organic phase	122626	3.278	6387.2	1.1
	More organic phase	1207845	3.015	4417	1.1

Results for LOD and LOQ

The LOD and LOQ values of RES and DHZ are given in Table 4.

Table 4: LOD and LOQ values of RES and DHZ.

Drug	LOD (µg/ml)	LOQ (µg/ml)
Reserpine	0.13	0.41
Dihydralazine	1.2	3.6

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Dihydralazine and Reserpine in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps.

Dihydralazine and Reserpine were freely soluble in ethanol, methanol and sparingly soluble in water. Buffer: Methanol: ACN (65:25:10v/v) was chosen as the mobile phase. The solvent system used in this method was economical.

The % RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Dihydralazine and Reserpine in bulk drug and in Pharmaceutical dosage forms.

BIBLIOGRAPHY

1. Dr. Kealey and P.J Haines. Analytical Chemistry. 1st Ed. Bios Publisher. 2002: 1-7. Braith Wait and F. J. Smith. Chromatographic Methods. 5th Ed. Kluwer Academic Publisher, 1996; 1-2.
2. Andrea Weston and Phyllisr Brown. HPLC Principle and Practice. 1st Ed. Academic press, 1997; 24-37.
3. Yuri Kazakevich and Rosario Lobrutto. HPLC for Pharmaceutical Scientists. 1st Ed. Wiley Interscience: A JohnWiley & Sons Inc. Publication, 2007; 15-23.
4. Chromatography (online). Url: http://en.wikipedia.org/wiki/Chromatography.
5. Meyer V.R. Practical High-Performance Liquid Chromatography. 4th Ed. England. John Wiley & Sons Ltd., 2004; 7-8.
6. Sahajwalla CG: A new drug development. Marcel Dekker Inc. New York, 2004; 141: 421–426.
7. Detectors used in HPLC (online). Url: http://wiki.answers.com/Q/What_detectors_are _used_in_HPLC.
8. Detectors (online). Url: http://hplc.chem.shu.edu/NEW/HPLC_Book/Detect ors/det_u vda.html.
9. Draft ICH Guidelines on Validation of Analytical Procedures Definitions and terminology. Federal Register. IFPMA. Switzerland, 1995; 60: 1126.
10. Saroj Kumar Raul, B.V.V Ravi kumar, Ajaya Kumar Pattnaik and Nagireddy Neelakanta Rao. a rp-hplc method development and validation for the estimation of dihydralazine in bulk and pharmaceutical dosage forms. Int J Pharm, 2013; 3(1): 116-121.
11. G. Santhosh, G. Nagasowjanya, A. Ajitha1, Dr. V. Uma Maheshwara Rao. Analytical method development and validation of simultaneous estimation of isosorbide dinitrate and hydralazine hcl in tablet dosage form by rp-hplc. International Journal of Pharmaceutical Research & Analysis, 2014; 4(5): 307-311.
12. Spurthy Kassey, Ravi Pratap Pulla, K. Vanitha Prakash. Development And Validation Of Stability Indicating Rphplc Method For Simultaneous Estimation Of Isosorbide Dinitrate And Hydralazine Hcl In Combined Pharmaceutical Dosage Form. IAJPS, 2014; 1(4): 219-229.