WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 10, 505-514. Research Article ISSN 2277–7105

METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION FOR PROPRANOLOL AND HYDRALAZINE HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY USING UV SPECTROSCOPY

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Article Received on
29 March 2018,
Revised on 18 April 2018,
Accepted on 09 May 2018
DOI: 10.20959/wjpr201810-12249

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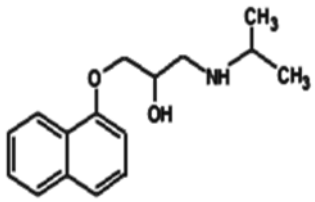
ABSTRACT

Applying a simple technique of simultaneous equation and area under curve method by spectrophotometric. The aim of this study was to develop and validate with accurate, precision, reproducible and economical for simultaneous estimation of propranolol and hydralazine in bulk and marketed formulation. Maximum absorbance was shows at two different wavelengths of 289 nm and 314 nm that are the absorption maximum values of propranolol and hydralazine hydrochloride respectively in distilled water and it also used for baseline correction. In these methods, linearity range was 10-60µg/mL and 7-42µg/mL respectively for propranolol and hydralazine. The percentage RSD value was found to be less than 2%. The method was validated according to the ICH guidelines with respect to their parameters like specificity, linearity, accuracy, precision and robustness. The obtained result of analyses has been validated statistically. The method developed can be for the routine analysis of propranolol and hydralazine from their combined dosage form.

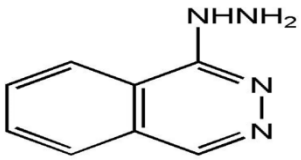
KEYWORD: Propranolol, Hydralazine, Simultaneous equation method and Area under curve, Spectrophotometric.

INTRODUCTION

Propranolol chemically (RS)-1(isopropylamino) 3-(1-naphthyloxy) propan-2-ol[1] is a nonselective beta blocker and is mainly used in the treatment of hypertension by blocking the action of epinephrine and norepinephrine on both β1 and β2 adrenergic receptors and also used in the management of hypertension, angina pectoris, myocardial infarction, migraine, glaucoma etc. Hydralazine (1-hydrazinylphthalazine, HDZ)[2-4] is a direct acting smooth muscle relaxant used to treat hypertension by acting as a vasodilator primarily in arteries and arterioles. Vasodilators act to decrease peripheral resistance, thereby lowering blood pressure and decreasing afterload. It has also clinical application in after heart valve replacement and in the treatment of chronic – resistant heart failure. It is widely used in combination with β-blocking drug (to balance the reflex tachycardia) and a diuretic (to decrease sodium retention) for the treatment of essential hypertension. Literature review reveals that few analytical method development and validation reported for propranolol individual and combination.[5-16] Hydralazine alone and combination[17-19] and however, there is no analytical method reported for method development and validation for simultaneous estimation of propranolol and Hydralazine hydrochloride in bulk and its formulation by UV spectroscopic method. Present work describes rapid, simple, accurate and reproducible UV spectroscopic method. The method validated in compliance with ICH guidelines.[20]



a.



b.

Fig no.1: a) Structure of Propranolol. b) Structure of Hydralazine.

MATERIALS AND METHODS

Chemicals and Reagents

Propranolol and Hydralazine were supplied as gift sample by D.K. Pharma Chem. Pvt. Ltd. (Maharashtra, India). The commercial formulation [Carbetazine], Nicholas Piramal India ltd Mumbai, Maharashtra] with label claim 40 mg/25mg per tablet was purchased from local pharmacy. Distilled water was used as a diluent for the preparation of drug samples.

Instrumentation

A shimadzu 1800 UV/VIS double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements processed by UV-probe. Single Pan Electronic balance (CONTECH, CA 223, India) was used for weighing purpose. Sonication of the solutions were carried out using an Ultrasonic Cleaning Bath (Spinco tech, India).

Experiments

Selection of diluent

Distilled water used as a solvent for developing spectral characteristics of the drugs. The solvent selection completed after assessing the solubility in different solvents like 0.01N HCl, methanol and ethanol. The drug showed complete solubility in Distilled water.

Preparation of standard stock solution

Accurately weighed 10 mg of Propranolol and Hydralazine standard substances and transferred into 10ml volumetric flask separately. Dissolved in distilled water and made up to the volume with distilled water (1mg/mL).

Selection of wavelength

For the selection of analytical wavelength, from the above standard stock solutions pipette out 0.1ml and transferred into 10ml volumetric flask, which contains 10ml of distilled water. Concentrations obtained 10µg/ml of respective standards and then observed at two different wavelengths of 289 nm for Propranolol and 314 nm for Hydralazine which shown fig no.2 of overlay spectra of both.

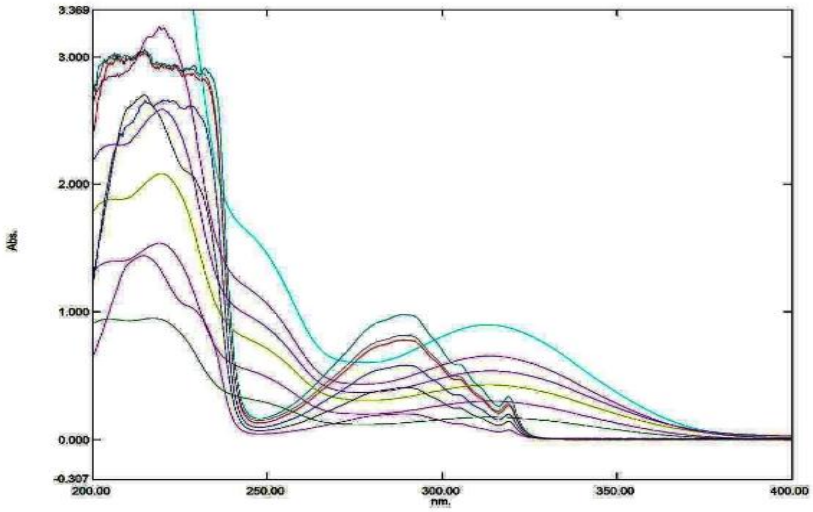


Fig 2: Overlay spectra of propranolol and Hydralazine hydrochloride.

Simultaneous Equation Method A

If a sample contain two absorbing drugs each of which absorbs at the λmax of the other, it may be possible to determine both drugs by the technique of simultaneous equation. Two wavelengths selected for the development of the simultaneous equations are 289 nm and 314 nm. The absorptivity values determined for propranolol at 298 and 316 are (ax1), and (ax2). For Hydralazine are (ay1), and (ay2) at 316 nm and 298 nm respectively. These values are means of six estimations. The absorbance and absorptivity at these wavelengths were substituted in equation 1 and 2 to obtain the concentration of both drugs. Obtained values shown in table 1.

Cx = (A2 ay1 – A1 ay2) / (ax2 ay1 – ax1 ay2) ……(1)

Cy = (A1 ax2 – A2 ax1) / (ax2 ay1 – ax1 ay2) ……. (2)

A1 and A2 = Absorbance of sample at λ1 and λ2

Cx and Cy = Concentrations of propranolol and Hydralazine in sample matrix.

ax1 and ax2 = Absorptivities of propranolol at λ1 and λ2

ay1 and ay2= Absorptivities of Hydralazine at λ1 and λ2

Table 1: simultaneous equation method results.

Drugs	289 λ nm	314 λ nm
Propranolol	0.573(ax_1)	0.289(ax_2)
Hydralazine	0.435(ay_1)	0.537(ay_2)
Marketed formulation	0.693(A_1)	0.521(A_2)

From the above values, concentration ratio between propranolol and hydralazine were found to be in the ratio of 1.08:0.619.

Area under Curve Method B

From the overlay spectra of drug area under the curve in the range of 286-292 nm for propranolol and 311-317nm for Hydralazine was selected for the analysis. The calibration curves for propranolol and hydralazine were prepared in the concentration range of 10-60 µg/ml and 7-42µg/ml at their respective AUC range. The absorbance values of the drug were determined at the selected AUC range. The absorbance is the ratio of area under the curve at selected wavelength ranges with the concentration of component in mg/ml. These absorbance values were the mean of six independent determinations.

RESULTS AND DISCUSSION

The UV spectra of Propranolol and Hydralazine hydrochloride, absorption maxima were obtained at 289nm and 314nm respectively. Propranolol and Hydralazine hydrochloride showed linearity in the concentration range of 10-60µg/ml and 7-42µg/ml. The proposed linearity ranges were obeys Beer-Lambert`s law. In table 2 shows, the statistical data obtained for the method A and method B. The quantitative results obtained and subjected to statistical analysis to find out standard deviation and standard error values. The relative standard deviation values are below indicating the precision of the methodology. The repeatability of the method was confirmed by the assay procedures with three different concentrations of three replicates each. The results obtained in repeatability test expresses the precision of the given method. The proposed method was validated as per the ICH guidelines.

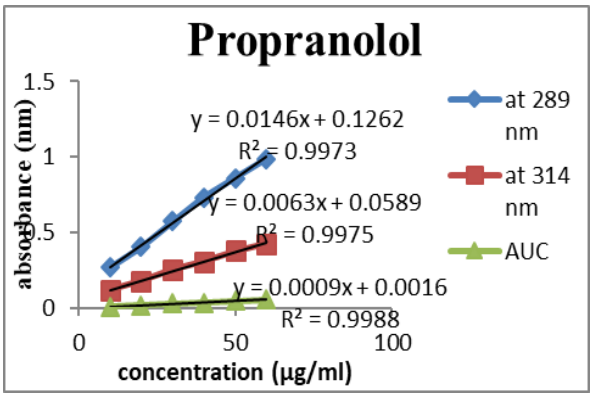
Table 2: Statistical data for the Method A and Method B Spectroscopic method for Propranolol and Hydralazine HCl.

Parameters	Method A				Method B	
	Propranolol		Hydralazine		Propranolol	Hydralazine
λmax (nm)	289	314	314	289	286-291	311-317
Linearity range (µg/ml)	10-60	10-60	7-42	7-42	10-60	7-42
Regression equation (Y*)	0.0146x+ 0.1262	0.0063x+ 0.0589	0.0174x+ 0.0546	0.0156x+ 0.0071	0.0009x+ 0.0016	0.0054x- 0.0059
Slope (m)	0.0146	0.0063	0.0174	0.0156	0.0009	0.0054
Intercept (c)	0.1262	0.1262	0.0546	0.0071	0.0016	-0.0059
Correlation coefficient (r2)	0.997	0.9975	0.999	0.9961	0.9988	0.9989
Precision Interday (%RSD)	0.25	0.48	0.164	0.35	1.64	0.75
Precision Intraday (%RSD)	0.30	0.44	0.23	0.37	1.29	0.65
LOD (µg/ml)	0.313	0.559	0.179	0.317	1.357	0.587
LOQ (µg/ml)	0.948	1.664	0.542	0.962	4.111	1.778

METHOD VALIDATION

LINEARITY

In order to find out linearity range of proposed UV-spectrophotometric methods, studies were carried out by plotting absorbance against concentrations of the analyte. A good linear relationship for method-A & method-B were observed between concentrations of propranolol and the corresponding absorbance. The linearity graphs were shown in fig 3 (a & b).



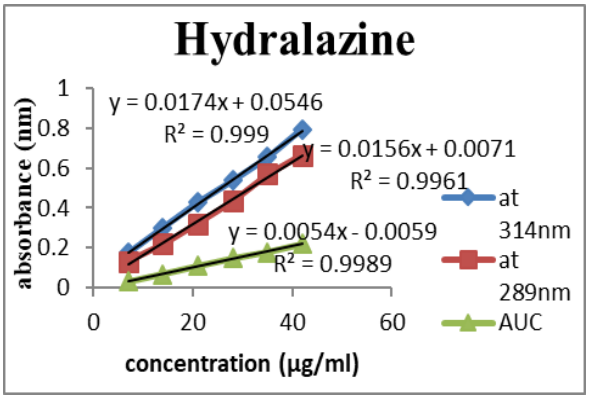


Fig 3a. Linearity graphs for Propranolol. Fig 3b. Linearity graphs for Hydralazine.

ACCURACY

To show the accuracy of this method by performing recovery studies. Here the recovery studies results were obtained by the procedure of triplet standard addition method at different concentration levels of 50%, 100% and 150%. By adding known amount of standard hydralazine and propranolol to pre analyzed samples and were subjected to the proposed Method A and Method B. Results of recovery studies are shown in table 3.

Table 3: Accuracy data.

Accuracy (%)	Method A				Method B	
	Hydralazine		Propranolol		Hydralazine	Propranolol
	% Recovery at 314nm	% Recovery at 289nm	% Recovery at 289nm	% Recovery at 314nm	% Recovery 311-317nm	% Recovery 286-292nm
50	99.7	99.8	101.2	101.3	100.7	99.9
100	100.0	100.0	100.3	99.9	99.8	100.3
150	100.7	101	100.0	99.41	100.9	100.8

PRECISION

The repeatability of the method was confirmed by the analysis of formulations was repeated for six times with the same concentration. The amount of each drug present in the tablet formulations was calculated. The % RSD and Confidence Interval were calculated. The intermediate precision of the method was confirmed by intraday and inter day analysis where the analysis of formulation was repeated three times in the same day and one time on three successive days. The amount of drugs was determined, %RSD and Confidence Interval were calculated. The result was tabulated in table 4(a&b).

Table 4a: Precision Data.

Method A								
S.No	Hydralazine at 314nm		Hydralazine at 289nm		Propranolol at 289nm		Propranolol at 314nm	
	Inter Day	Intra Day	Inter Day	Intra Day	Inter Day	Intra Day	Inter Day	Intra Day
1	0.574	0.573	0.433	0.435	0.558	0.558	0.242	0.243
2	0.575	0.570	0.435	0.432	0.556	0.556	0.243	0.243
3	0.573	0.571	0.431	0.430	0.556	0.556	0.241	0.241
4	0.574	0.572	0.432	0.432	0.557	0.559	0.242	0.242
5	0.572	0.573	0.433	0.433	0.558	0.558	0.241	0.241
6	0.574	0.574	0.432	0.434	0.554	0.554	0.243	0.243
Mean	0.573667	0.572167	0.4325	0.432667	0.5565	0.556833	0.241833	0.242167
Std.Dev	0.000943	0.001344	0.0015	0.001599	0.001384	0.001675	0.001067	0.001155
%RSD	0.16	0.24	0.35	0.37	0.25	0.30	0.44	0.48

Table 4b: Precision Data

Method B				
S.No	Hydralazine at 311-317nm		Hydralazine at 286-292nm	
	Inter Day	Intra Day	Inter Day	Intra Day
1	0.148	0.148	0.029	0.029
2	0.146	0.147	0.029	0.028
3	0.147	0.147	0.029	0.029
4	0.148	0.146	0.029	0.029
5	0.146	0.145	0.028	0.029
6	0.145	0.146	0.028	0.029
Mean	0.146666667	0.1465	0.028667	0.028833333
Std.Dev	0.001105542	0.000957427	0.000471	0.000372678
%RSD	0.75	0.65	1.64	1.29

Detection of limits (LOD&LOQ)

It is the lowest amount of analyte in a sample that can be detect but not necessarily quantities as an exact value under the stated, experimental conclusions. The detection limit is usually expressed as the concentration of analyte. The standard deviation and response of the slope- LOD=3.3 * standard deviation (σ)/ s. The quantitation limit of an analytical procedure is the lowest amount of an analyte of a sample, which can be quantitatively determined with suitable precision and accuracy. The standard deviation and response of the slope- LOQ=10* standard deviation (σ)/ s. The results of LOD&LOQ were tabulated in table 2.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness data were shows in table 5.

Table 5: Robustness data.

S.No.	Robust Condition	Hydralazine		Propranolol	
		Parameter	%RSD	Parameter	%RSD
1	Wave length ± 3 nm	311nm	0.15	286nm	0.39
2		314nm	0.23	289nm	0.27
3		317nm	0.08	291nm	0.31

CONCLUSION

The proposed method was simple, precise, accurate, rugged and economical and they were confirmed by their obtained low %RSD values. The percentage recovery indicates that the excipients used in formulation doesn't interfering during analysis of formulation. Hence the developed method can be applied for the routine quality control analysis of hydralazine and propranolol in bulk and combined tablet dosage form.

ACKNOWLEDGEMENT

I am very thankful to Director, JNTUA-OTPRI, Ananthapuramu for providing the laboratory facilities, chemicals to carryout entire research work. The authors are thankful to D.K.Pharma Chem. Pvt.Ltd., Mumbai for supplying the gift samples of hydralazine and propranolol.

REFERENCES

1. Indian Pharmacopoeia. Ghaziabad: The Indian pharmacopoeia commission, 2007; 3: 128-34.
2. Indian Pharmacopoeia, Ghaziabad: The Indian pharmacopoeia commission, 2010; 3: 1987-9
3. The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Whitehouse Station, NJ: Merck and Co., Inc, 2001; 850.
4. https://www.drugbank.ca/drugs/DB01275.
5. Becket AH, Stenlake JB. Practical Pharmaceutical Chemistry. Part –II.4th edition. CBS Publishers and Distributors: New Delhi, 2007.
6. Jasmine Chaudhary, Akash Jain and Vipin Saini, Analytical Method Development and Validation for the Simultaneous estimation of Alprazolam and Propranolol in their combined dosage form, International Journal of Drug Delivery, 2012; 4: 310-315.
7. P V Devi Swapna, Paramita Das, S H.Chetan, Jitender Reddy, Analytical Method Development And Validation For The Simultaneous Estimation Of Propranolol Hydrochloride And Etizolam In Their Combined Dosage Form By Vierodt's Method,

International Journal of Pharmaceutical Biological and Chemical Sciences, 2014; 3(2): 46-50.

8. S. J. Daharwal, Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Diazepam and Propranolol in Bulk Drug and its formulations, Asian Journal of Pharmaceutical Analysis, 2013; 1(3): 20-23.
9. Lakshmi Narasimham Y.S.*, Barhate V.D, Development And Validation Of Stability Indicating Uplc Method For The Simultaneous Determination Of Beta-Blockers And Diuretic Drugs In Pharmaceutical Dosage Forms, Journal of Chemical Metrology, 2010; 4(1): 1-20.
10. Y. Shalini, et al, Method Development and Validation for Simultaneous Estimation of Propranolol Hydrochloride and Flunarizine Dihydrochloride in Bulk and Pharmaceutical Dosage Form by RP-HPLC, International Research Journal of Pharmaceutical and Applied Sciences, 2012; 2(5): 143-148.
11. Anil K. Singh, Erika R. M. Kedor-Hackmann, Maria Inês R. M. Santoro, Enantiomeric Separation And Quantitative Determination Of Propranolol Enantiomers In Pharmaceutical Preparations By Chiral Liquid chromatography, Revista Brasileira de Ciências Farmacêuticas Brazilian Journal of Pharmaceutical Sciences, 2004; 40(3): 301-308.
12. Yadav Priya, Majee Chandana, Method Development and Validation for Quantification of Propranolol HCl in Pharmaceutical Dosage form by RP-UPLC, International Journal of PharmTech Research, 2014-2015; 7(1): 197-203.
13. Payal Kate, et al, Stability indicating RP-HPLC method development and validation of Etizolam and Propranolol hydrochloride in pharmaceutical dosage form, World Journal of Pharmaceutical Sciences, 2015; 3(6): 1113-1124.
14. Bhadresh V. Savaj, Ashutosh kumar Patidar, Hashumati A. Raj, Simultaneous determination of Propranolol hydrochloride and Hydrochlorothiazide in Tablets formulation using spectrophotometric technique (Simultaneous Equation Method), International Journal of Pharma Sciences and Research, 2015; 6(2): 245-251.
15. S. M. Korani, D. B. Doshi, Development and Validation of Stability Indicating HPTLC Method for Simultaneous Estimation of Propranolol Hydrochloride and Flunarizine Dihydrochloride, International Journal of Pharma Research & Review, 2016; 5(7): 1-8.
16. Xiaolei Tang, et al, Development and Validation of HPLC Methods for the Determination of Propranolol Hydrochloride and Hydrochlorothiazide Related Substances in

Combination Tablets, International Journal of Drug Development and Research, 2017; 9: 24-29.

17. Liu Y, Li H, Luo H, Lin Z, Luo W. LC–MS–MS Method for Quantification of Hydralazine in BALB/C Mouse Plasma and Brain: Application to Pharmacokinetic Study. Chromatographia, 2011; 73: 1183-1188.
18. Napa.Delhiraj, Sockalingam.Anbazhagan, Validated Liquid Chromatographic Method For The Estimation Of Antihypertensive Mixture In Pharmaceutical Dosage Forms, World Journal Of Pharmacy And Pharmaceutical Sciences, 2014; 3(6): 1916-1927.
19. Ajit Anerao, et al. Development And Validation Of Related Substances Method By Hplc For Analysis Of Hydralazine Hydrochloride, World Journal Of Pharmacy And Pharmaceutical Sciences, 2015; 4(11): 1944-1965.
20. ICH Q 2 (R1) Text on validation of analytical procedures, International Conference on Harmonization tripartite guidelines, adapted 27 June 1995.