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Research Article

DEVELOPMENT AND VALIDATION OF A SIMPLE UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF URAPIDIL HYDROCHLORIDE BOTH IN BULK AND PHARMACEUTICAL FORMULATION

***S. Navgire[1], A. Ghadge[2], S. Gurav[2], S. G. Killedar[3] and S. J. Nadaf[4]**
[1]*Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University,*
[2] *Government College of Pharmacy, Karad-415110, Maharashtra, India*
[3]*Bharati Vidyapeeth College of Pharmacy, Kolhapur- Maharashtra, India*
[4]*Adarsh College of Pharmacy, Bhavaninagar, Vita- Maharashtra, India*
**Author for Correspondence*

ABSTRACT
Background: Urapidil, a symphatolytic anti hypertensive drug is being developed for the management of mild to severe hypertension, with little sway on heart rate. *Aim:* In present work an effort has been made to develop an accurate, simple and precise UV Spectrophotometric method for estimation of urapidil hydrochloride both in bulk and pharmaceutical formulation. *Materials and Methods:* Work was carried out by preparing internal standard of various concentrations and further detections were carried out at analytical wavelength (245 nm). Method was further validated as per International conference on Harmonisation (ICH) guidelines for linearity, accuracy and precision. *Results:* The concentration of urapidil over a range of 2-14 µg/ml, obeys Beers law. The correlation co-efficient for urapidil was found to be 0.998 and the % relative standard deviation (RSD) was within specification limits (< 2). Percent Recovery estimated was found to be 98.86 ± 0.3725. *Conclusion:* On the basis of validation report it can be concluded that method is accurate, precise and shows satisfactory linearity. Hence, this simple, precise, accurate and economic method can be applied in regular laboratory analysis.

Keywords: *Urapidil, Recovery, Validation, Antihypertensive*

INTRODUCTION
Urapidil (Figure 1) is a potent anti hypertensive compound without serious side effects. Its action is mainly due to a postsynaptic α_1- adrenoceptor antagonism that inhibits the vasoconstrictive action of catecholamines and reduces blood pressure by decreasing peripheral vascular resistance. Urapidil also has an agonistic effect on central 5-HT1A receptors and lowers blood pressure by preventing the stimulation of baroreceptors (Wang and Yuan, 1993).

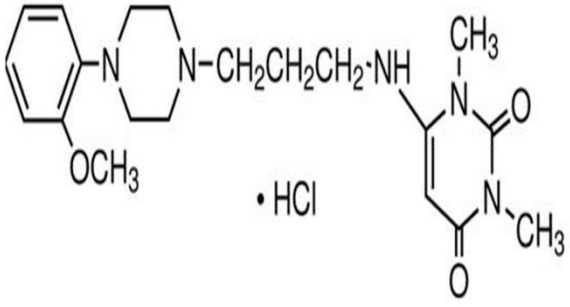

Figure 1: Structure of Urapidil Hydrochloride

Since Urapidil is a widely used drug, an effective method for its analysis is highly desirable. Current methods include high performance liquid chromatography (HPLC) (Zech and Huber, 1986; Baker, 1987;

Huber and Zech, 1988), chemiluminescence (CL) (Lang *et al.*, 2003) and fluorescence spectrophotometry, mass spectroscopy (Jianshe *et al.*, 2011; Ambavaram *et al.*, 2013). These methods are either too complicated, or of low sensitivity.

So far to our present knowledge, UV spectrophotometric analytical method is not available in literature for analysis of Urapidil hydrochloride in pharmaceutical dosage form oras bulk drug sample. It was sensed necessary to develop a simple, precise, easy, and economic as well as rapidmethod for quantitative determination of Urapidil hydrochloride. The current research work deals with development UV spectrophotometric method using distilled water for the determination of Urapidil hydrochloride in pharmaceutical dosage form or as bulk drug sample and its validation as per International Conference on Harmonisation (ICH) guidelines (ICH).

MATERIALS AND METHODS

Apparatus

A Shimadzu UV–visible spectrophotometer (UV mini-1700, Shimadzu Corporation, Kyoto, Japan) was used for all absorbance measurements with matched quartz cells.

Materials

Urapidil hydrochloride in the form of Urapidil hydrochloride powder was provided by Lupin pharmaceuticals Pune, which was used as the reference standard. All the chemicals and reagents used were of analytical or HPLC grade.

Methods

Preparation of Standard Stock Solution

Accurately weighed 20 mg of Urapidil hydrochloride was transferred into 100ml volumetric flask, dissolved in 50ml distilled water by shaking it for 10min. The volume was made up to mark with distilled water to obtain stock solution of 200µg/ml concentration.

Selection of Wavelength for Analysis of Urapidil Hydrochloride

Appropriate volume 5 ml of standard stock was transferred into 100ml volumetric flask, diluted to mark with distilled water to give concentration of 10µg/ml. The resulting Solution was scanned from UV range (200-400nm). In spectrum Urapidil hydrochloride showed absorbance maximum at 269nm (Figure 2).

Validation of Method

The method was validated in terms of linearity, accuracy, precision and ruggedness as per ICH guidelines.

Linearity Study

Aliquots of 1–7 ml portion of stock solutions were transferred into series of 100ml volumetric flasks and further volume was made up to mark with distilled water, to get concentrations 2, 4, 6, 8, 10, 12 and 14 µg/ml, respectively. All the Solutions were scanned in the range of 200–400 nm against blank. The absorption maxima were found to be at 269 nm against blank. Further calibration curve was plotted using the data.

Accuracy

To the preanalysed sample solutions, a known amount of standard stock solution was added at different levels, i.e.80%, 100% and 120%. Solutions were reanalysed by the proposed method.

Intra-Day Precision (Repeatability) and Inter-Day Precision Study (Intermediate Precision)

Precision of the method was studied as intra-day and inter-day variations. Intra-day precision was determined by analysing 6, 8, and 10 µg/ml of Urapidil hydrochloride solutions for three times in the same day. Inter-day precision was determined by analysing 6, 8, and 10µg/ml of Urapidil hydrochloride solutions for three days over the period of week.

Sensitivity

The sensitivity of measurements of Urapidil hydrochlorideby the use of the proposed method was estimated in terms of the limit of detection (LOD) and limit of quantitation (LOQ).

LOD and LOQ were calculated using equation LOD = 3.3×N/B and LOQ = 10 ×N/B, where ‘N’ is the standard deviation of absorbance of sample (n=3), ‘B ‘is the slope of the corresponding standard calibration curve.

Determination of Urapidil Hydrochloride in Bulk

Accurately weighed 10mg of Urapidil hydrochloride was transferred into a 100 ml volumetric flask containing 50 ml distilled water, and the volume was made up to the mark using the same. Appropriate volume 1 ml of this solution was transferred to a 10ml volumetric flask, and the volume was adjusted to the mark using distilled water. The resulting solution was scanned on a spectrophotometer in UV range of 200–400 nm. The concentration of the drug was calculated from linear regression equations.

Application of the Proposed Method for Pharmaceutical Formulation

Six tablets were weighed and powdered. The amount of tablet powder equivalent to 10 mg of Urapidil hydrochloride was weighed accurately and transferred into 100ml volumetric flask then 50ml of distilled water was added and kept for 15 min with frequent shaking and volume was made up to mark with distilled water. Then, the solution was filtered through the Whattman filter paper. The filtrate was diluted suitably with distilled water to get the solution of 10µg/ml concentration. The absorbance was measured against solution blank. The drug content of the preparation was calculated using standard calibration curve.

RESULTS AND DISCUSSION

Method Validation

The proposed method was validated as per the ICH guidelines. The solutions of the drugs were preparedas per the earlier adopted procedure given in the experiment.

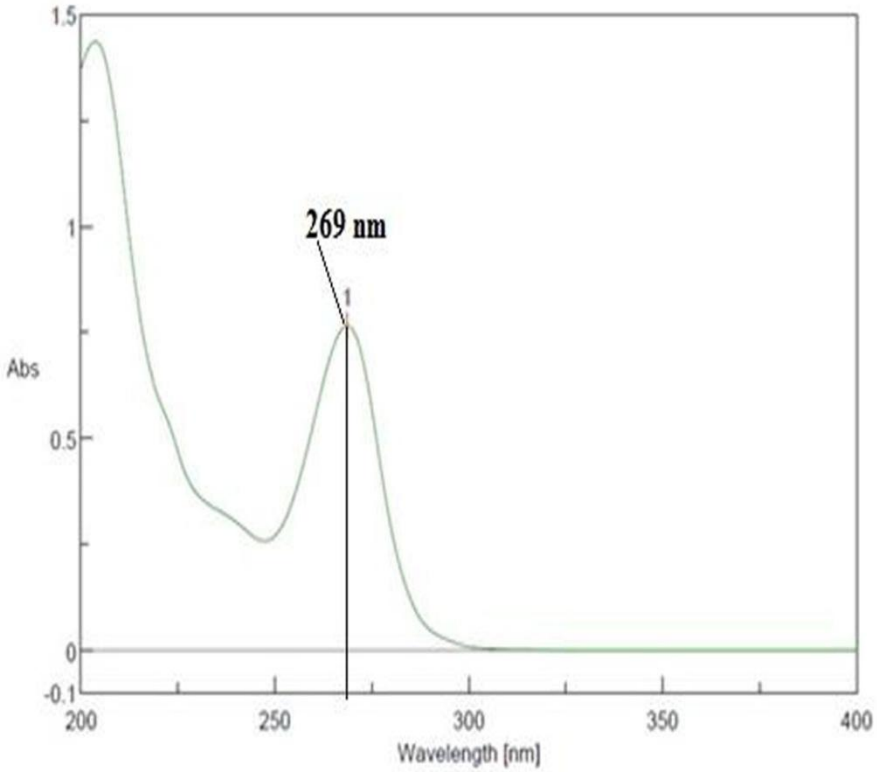

Figure 2: UV Spectra of Urapidil Hydrochloride at 269 nm

Linearity Study

The linear regression data for the calibration curves has shown linear relationship over the concentration range of 2- 14µg/ml for Urapidil hydrochloride. Linear regression equation was found to be Y = 0.082X + 0.004 (R^2= 0.998). Absorbance of the solution at different concentration as reported in Table 1 and figure 3.

Table 1: Linearity Study of Urapidil Hydrochloride

Sr. No.	Concentration (µg/ml)	Absorbance (Average)
1	2	0.1847
2	4	0.3346
3	6	0.4976
4	8	0.6588
5	10	0.8098
6	12	0.9816
7	14	1.1822

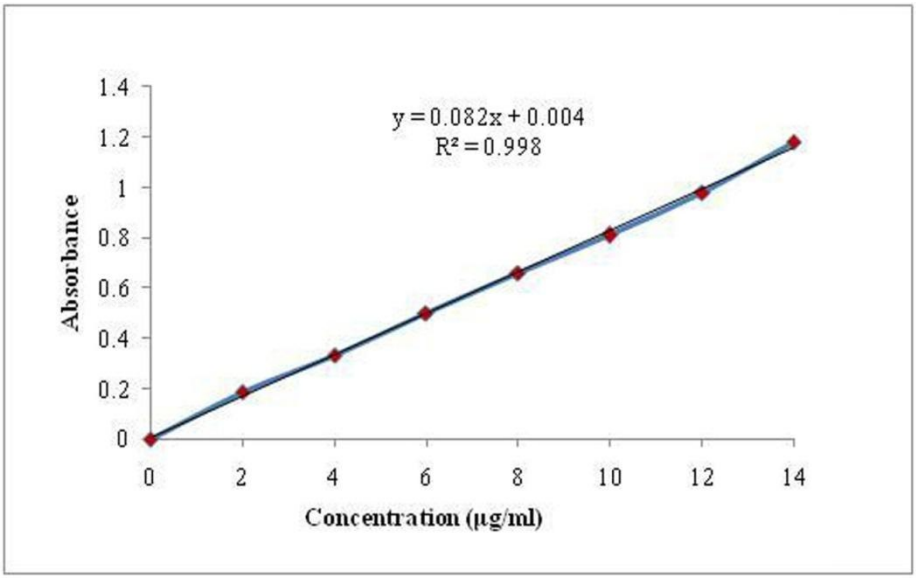

Figure 3: Calibration Curve of Urapidil Hydrochloride at 269 nm

Accuracy

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in powdered tablets. The recovery was performed at three levels, 80, 100, and 120% of Urapidil hydrochloride standard concentration. The recovery samples were prepared in afore mentioned procedure. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for Urapidil hydrochloride are given in Table 2.

Table 2: Summary of Recovery Study*

Reanalyzed Sample (µg/ml)	Amount of Drug Added (µg/ml)	Amount Recovered (µg/ml)	% Recovery
10µg/ml	0	9.86	98.65
	8	17.96	99.73
	10	19.76	98.79
	12	21.84	99.27

* Indicates ± SD (n=3)

Precision

The precision of the developed method was expressed in terms of the % relative standard deviation (%RSD). These results shown reproducibility of the assay. The (%RSD) values found to be less than 1

that indicates this method precise for the determination of both the drugs in bulk as well as in the pharmaceutical formulation (Table 3).

Table 3: Summary of intra -day and inter-day precision study

	Intra -Day Precision (n=3)				Inter-Day Precision (n=3)			
Conc. (µg/ml)	**Absorbance (Mean ± SD)**	**%RSD**	**Conc. Found (µg/ml)**	**Average Potency %**	**Absorbance (Mean ± SD)**	**%RSD**	**Conc. Found (µg/ml)**	**Average Potency %**
8	0.4969 ± 0.00030	0.06	6.01	100.16	0.4966 ± 0.00052	0.105	6.0	100.00
10	0.6585 ± 0.00020	0.03	7.98	99.77	0.6540 ± 0.00608	0.93	7.92	99.08
12	0.8098 ± 0.00015	0.019	9.27	98.27	0.8082 ± 0.00083	0.103	9.80	98.07

* Indicates ± SD (n=3); RSD- relative standard deviation

Sensitivity

The linearity equation was found to be Y = 0.082X + 0.004 (R^2= 0.998). The LOD and LOQ for Urapidil hydrochloride were found to be 0.108 µg/ml and 0.32 µg/ml.

Determination of Urapidil Hydrochloride in Bulk

The concentrations of the drug were calculated from linear regression equation. The % average amount of drug was found to be 98.86% (Table 4).

Table 4: Analysis of Urapidil Hydrochloride in Bulk

Concentration (µg/ml)	Amount Found (µg/ml)	Amount Found (%)
10	9.85	98.51
	9.86	98.60
	9.85	98.58
	9.88	98.88
	9.92	99.24
	9.93	99.39
Mean ± SD	9.88 ± 0.0354	98.86 ± 0.3725
% RSD	0.358	0.377

Application of the Proposed Method for Pharmaceutical Formulation

The spectrum was recorded at 269 nm. The concentration of the drug was calculated from the linear regression equation. The % Average amount of drug was found to be 98.51% (Table 5).

Table 5: Analysis of Urapidil Hydrochloride Formulation

Concentration (µg/ml)	Amount Found (µg/ml)	Amount Found (%)
10	9.82	98.26
	9.85	98.51
	9.82	98.23
	9.85	98.58
	9.89	98.90
	9.86	98.60
Mean ± SD	9.84 ± 0.026	98.51 ± 0.247
% RSD	0.264	0.251

Conclusion

The UV spectrometric technique is simple, accurate, precise, reproducible, and sensitive. The UV method has been developed for the quantification of Urapidil hydrochloride in tablet formulation. The validation procedure confirms that this is an appropriate method for their quantification in the formulations. It is also used in the routine analysis of the formulations containing this entire compound.

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