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VALIDATED UV SPECTROPHOTOMETRIC METHOD FOR *IN-VITRO* DISSOLUTION STUDIES IN PHOSPHATE BUFFER pH 7.4

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ABSTRACT: A simple, accurate, and economical, least time-consuming method has been developed for Losartan potassium (LP) by using UV spectrophotometer. This method was developed using phosphate buffer solution pH 7.4 (PBS) for quantifying the amount of LP released from the colon-specific formulations. The wavelength maximum (λ_{max}) of LP in phosphate buffer pH 7.4 was found to be 228.5 nm. The developed UV spectroscopic method exhibited linearity in the range of 2-10 µg/mL with a correlation coefficient (R2) value of 0.999 and was validated with respect to linearity, specificity, accuracy (recovery), and precision. These parameters were determined according to International Conference on Harmonization (ICH) guidelines. Results of the analysis were validated statistically and by recovery studies, which proved the suitability of the developed method for the routine estimation of LP in colon-specific control release formulation. Hence, the reported method for the estimation of LP from the colon-specific formulations was simple, accurate, and least time-consuming.

INTRODUCTION: Losartan potassium, is chemically 2-butyl-4-chloro-1-[[2'-(1H-tetrazol-5-yl) [1,1'-biphenyl]-4-yl]methyl]-1H imidazole -5-methanol monopotassium salt. LP is categorized as Angiotensin-II receptor antagonist. It is a colorless, crystalline powder with excellent flow properties [1]. LP is a frequently prescribed drug for the management of Hypertension (HTN). The hypotensive effect is observed due to its Angiotensin II receptor (AT1) antagonistic property [2]. Furthermore, LP, together with its active carboxylic acid metabolite, cumulatively accounts for the antagonistic action on Angiotensin II receptor [3, 4].

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LP is a Biopharmaceutics Classification System (BCS) Class III drug being freely soluble in water. It is soluble in alcohols and slightly soluble in common organic solvents [5]. The structural formula of LP is shown underneath **Fig. 1**.

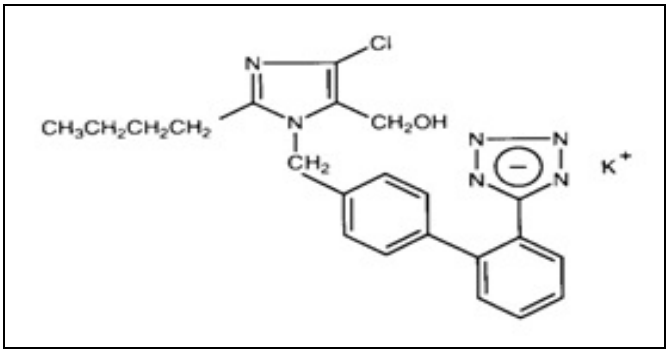

FIG. 1: STRUCTURE OF LP

The United States Pharmacopoeia (USP) endorses the use of HPLC for the estimation of LP in pharmaceutical samples 6. Many other analytical methods for LP determination alone or in

combination have been reported like UV spectrophotometry [7, 8], HPLC [10, 11], HPTLC [12, 13], electrochemical [14, 15].

The present work describes the development of validated UV spectro-photometric method for quantifying the amount of in-vitro LP release in PBS from colon-specific formulations [16].

MATERIALS AND METHODS: Losartan potassium was obtained as a generous gift sample by ZIM Laboratories Nagpur, India. Disodium hydrogen phosphate, potassium dihydrogen phosphate, and sodium chloride were procured from CDH, New Delhi, India. All other reagents used were of analytical grade.

Instrument: A double beam UV-visible spectro-photometer (Lab India 3200) having matched pair of 1 cm quartz cells was employed for analytical estimations.

Preparation of PBS pH 7.4: PBS was prepared by dissolving 2.38 g of disodium hydrogen phosphate, 0.19 g of potassium dihydrogen phosphate, and 8.0 g of sodium chloride in sufficient water to produce 1000 mL, pH was adjusted as per requirements [17].

Preparation of LP Standard Stock Solution: Standard stock solution of LP was prepared by accurately weighing 100 mg of drug and transferred into 100 mL volumetric flask. The drug was dissolved in PBS, and final volume was made with PBS to obtain the concentration of 1000 µg/mL [18].

Preparation of LP Working Dilutions: The working standard solution was prepared by taking 10 mL of standard stock solution in 100 mL volumetric flask. The volume was made up with PBS to obtain the concentration of 100 100 µg/mL [18, 19].

Determination of Wavelength Maximum (λ_{max}): LP standard concentration (6 µg/mL) was prepared in PBS and UV spectrum was taken using double beam UV-Visible Spectrophotometer (Lab India 3200) for the wavelength region of 200-400 nm and the wavelength maximum (λ_{max}) was found to be 228.5 nm **Fig. 2**.

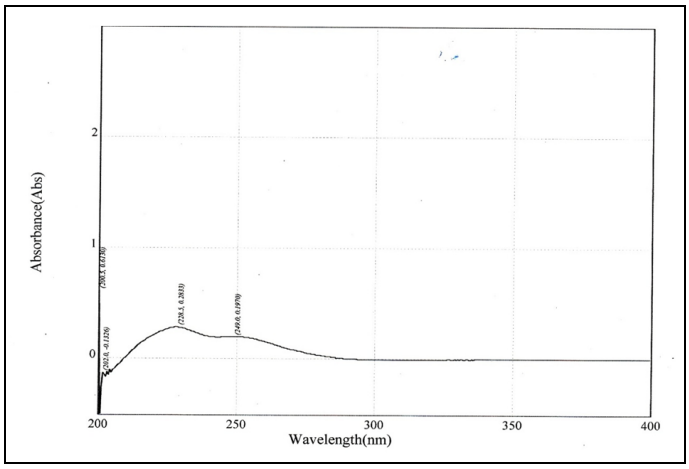

FIG. 2: UV SPECTRA OF LP SHOWING λ_{max} AT 228.5 nm IN PBS

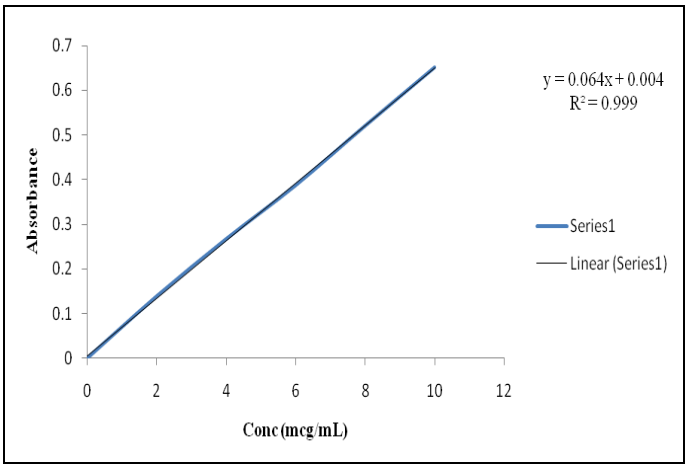

FIG. 3: STANDARD CALIBRATION CURVE OF LP IN PBS

Calibration Curve of LP in Phosphate Buffer Solution (pH 7.4): Aliquots with different volumes were withdrawn from working dilution into 10 mL volumetric flasks which were subsequently diluted with PBS (pH 7.4), in order to get standard drug concentrations between 2-10 µg/mL.

The respective absorbance of these LP dilutions was estimated at 228.5 nm [20]. A calibration curve was plotted between concentration (x-axis) and absorbance (y-axis) shown in **Fig. 3**.

Validation Procedure:

Linearity or Range: Working solution, 100 µg/ml was further diluted with PBS to get the final concentration in the range 2-10 µg/ml solutions.

The respective absorbance from the different concentrations (2-10 µg/ml) was measured at 228.5 nm. The calibration curve was constructed by plotting absorbance versus concentration, and the linear range was analyzed by regression equation [21].

Precision: The precision was expressed either for intraday or inter-day analysis as percent relative standard deviations.

The intraday study was conducted with a concentration of replicates of LP on the same day three times.

In the inter-day study, the concentration of the drug was analyzed on three successive days to get an insight of variable laboratory conditions on different days [21].

Repeatability: Repeatability study was conducted by analyzing the standard drug concentration for minimum in triplicate, and the percent relative standard deviation was calculated [21].

Accuracy or % Recovery: Recovery studies were performed to analyze the accuracy of the method. Different aliquots were taken to produce concentration levels at 80%, 100%, and 120% of standard test concentration.

The analysis of the sample was done in triplicate for each level. Percent recovery was then calculated as mentioned in the equation below [20].

$$\% \text{ Recovery} = A\text{-}B / C \times 100 \quad \ldots\ldots (1)$$

Where, A= amount of drug estimated in totality, B= amount of drug found on a pre-analyzed basis, C= amount of pure drug added to a formulation.

Limit of Detection (LOD): The Limit of Detection (LOD) is the smallest concentration of the analyte that can be measured. LOD was estimated employing the formula mentioned underneath [21].

$$LOD = 3.3 \times s / x \quad \ldots\ldots (2)$$

Where, X = slope of the standard curve, S = standard deviation of the response.

Limit of Quantification (LOQ): The Limit of Quantification (LOQ) is the response by a minimum quantity of the analyte that can be accurately quantified. LOQ was estimated employing the formula mentioned underneath [21].

$$LOQ = 10 \times s/ x \quad \ldots\ldots (3)$$

Where, X = slope of a calibration curve, X = standard deviation of the response.

% RSD: % RSD values were determined by using the formula based on the SD of response and mean of the response.

The % R.S.D. values found to be less than 2, indicating that the proposed method is precise [20, 22].

$$\% \text{ RSD} = \text{SD of Response} / \text{Mean of Response} \times 100 \quad \ldots\ldots (4)$$

RESULTS AND DISCUSSION:

Linearity or Range: The standard aliquots in the concentration range of (2-10 µg/ml) were analyzed for the absorbance at a wavelength maximum of 228.5 nm. The LP standard dilutions exhibited absorbance in the range 0.1392-0.6522.

The standard dilutions in this range were found to obey Beer-Lambert's law with regression of 0.999.

The values of Limit of detection (LOD) and limit of quantification (LOQ) for the assay have been given in **Table 1**.

TABLE 1: OPTICAL PARAMETERS OF LP

S. no.	Parameters	In PBS
1	Absorbance maximum (λ_{max}) in nm	228.5
2	Beer's Law Limit (µg/mL)	2-10
3	Equation	Y=0.0647x+0.0046
4	Slope	0.0647
5	Intercept	0.0046
6	Regression coefficient	0.999
7	LOD (µg/mL)	0.2001
8	LOQ (µg/mL)	0.6710

Accuracy or % Recovery: The concentration of recovery was found to be in the range of 10.70-13.26, and % RSD value was 0.07-0.885 respectively, shown in **Table 2**.

TABLE 2: RESULTS FOR DETERMINATION OF RECOVERY

Amount taken	Amount added % µg/mL		Conc. µg/mL	Absorbance			Conc. Found µg/mL	Mean	St.dev	%RSD
6	80	4.8	10.8	0.697	0.698	0.697	10.70	0.697	0.0005	0.07
6	100	6	12	0.798	0.789	0.757	12.02	0.781	0.0219	0.86
6	120	7.2	13.2	0.855	0.859	0.870	13.26	0.861	0.0076	0.88

Results for Determination of Intraday Precision: The % RSD for intraday precision for the concentration 2 µg/mL, 4 µg/mL and 6 µg/mL was determined at 10 am, 1 pm, and 4 pm the results have been shown in **Table 3**. Results obtained from the analytical method had a good intraday precision.

TABLE 3: RESULTS FOR DETERMINATION OF INTRADAY PRECISION

Timing	2 µg/mL Mean	Conc. Found (µg/mL)	SD	% RSD	4µg/mL Mean	Conc. Found (µg/mL)	SD	% RSD	6µg/L Mean	Conc. Found (µg/mL)	6µg/mL SD	% RSD
10 AM	0.142	2.10	0.0005	0.30	0.264	4.01	0.0004	0.16	0.387	5.91	0.0001	0.33
1PM	0.145	2.17	0.0027	1.92	0.267	4.03	0.0006	0.24	0.391	5.90	0.0053	1.36
4 PM	0.147	2.20	0.0021	1.43	0.268	4.00	0.0011	0.42	0.391	5.95	0.0051	1.32

Results for Determination of Interday Precision: The % RSD for interday precision for the concentration 2 µg/mL, 4 µg/mL and 6 µg/mL was according to three days analysis and the results have been shown in **Table 4**. Results obtained from the analytical method had a good interday precision.

TABLE 4: RESULTS FOR DETERMINATION OF INTERDAY PRECISION

Day	2µg/ml Mean	Conc. Found (µg/mL)	SD	% RSD	4µg/mL Mean	Conc. Found (µg/mL)	SD	% RSD	6µg/mL Mean	Conc. Found (µg/mL)	6µg/mL SD	% RSD
1	0.142	2.10	0.0005	0.37	0.264	4.01	0.0004	0.16	0.387	5.91	0.0001	0.39
2	0.143	2.00	0.0012	0.86	0.268	4.07	0.0009	0.35	0.388	5.96	0.0005	0.14
3	0.143	2.12	0.0005	0.38	0.269	4.03	0.0007	0.26	0.388	5.97	0.0055	0.14

Results for Determination of Repeatability: The repeatability of the proposed method was assessed by analyzing LP at 6 µg/mL concentration in triplicate. Results of repeatability analysis were expressed in terms of % RSD and found to be 0.195, 0.146, and 0.144, as shown in **Table 5**.

TABLE 5: RESULTS FOR DETERMINATION OF REPEATABILITY

Day	6µg/mL Mean	Conc. Found (µg/mL)	SD	%RSD
10 am	0.388	5.92	0.0007	0.19
1 pm	0388	5.92	0.0005	0.14
4 pm	0.389	5.94	0.0005	0.14

CONCLUSION: The experimental findings and the statistical parameters are in agreement that the developed UV spectrophotometric method of LP in PBS is simple, quick, specific, accurate, and precise. Furthermore, the analytical method developed has been analyzed on various parameters to confirm stability like specificity, linearity, accuracy, precision and robustness.

Hence this method can be used for the routine analysis of LP as API in pharmaceutical formulations designed for colon-specific release.

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CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

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