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Extractive Spectrophotometric Method For Simultaneous Determination Of Losartan Potassium And Atenolol In Bulk And In Pharmaceutical Dosage Form

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Abstract: A simple, accurate and precise extractive spectrophotometric method was developed for the Simultaneous estimation ofLosartanPotassium and Atenolol in both pure and pharmaceutical dosage form. The method was based on the formation of colored complex by Losartan Poatassium and Atenolol withreagents like ferroin Solution and Methyl orangerespectively in Phosphate buffer PH 7.0. The linearity rangeof LosartanPotassium and Atenolol were found to be 25-300 µg/ml and 50-1400 µg/mlrespectively. The ion-pair complex formed was quantitatively extracted under the experimentalconditions with chloroform and the absorbances of the organic layers were measured at 571.0nm and 426.0 nm for Losartan Potassium and Atenolol respectively. The correlation coefficient (r^2) for Losartan Potassium and Atenolol were found to be 0.9975 and 0.9974, respectively. The method wasstatistically evaluated and was found to be precise and accurate. This methodisvalidatedas per the guidelines of ICH. The method is applied for the determination of drugs in commercial tablet.

Keywords: Losartan Potassium and Atenolol, Extractive spectrophotometric method, Ferroin Solution andMethyl orange indicator.

INTRODUCTION:

Losartan potassium (LSK) is chemically Monopotassium salt of 2-butyl-4-chloro-1-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4yl]methyl]-1*H*-imidazole-5-methanol (Fig.1) used asantihypertensive drug by inhibiting Angiotensin II receptor[1-5]. LSK and its tablet dosage form is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) and United state Pharmacopoeia (USP). It is estimated by potentiometric titration and chromatographic method as per IP, BP & USP[6-8].Literature review also reveals that HPLC[10-12], HPTLC[13], UV[14-18]spectrophotometric and extractive spectrophotometric[19-25] methods have been reported for the estimation of LSK in pharmaceutical dosage forms.Atenolol (ATN) is chemically (*RS*)-4-(2-hydroxy-3-Isopropylamino propoxy)phenyl acetamide (Fig. 2) used as antianginal, antihypertensive drug[1-5]. It is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) and United state Pharmacopoeia (USP). It is estimated by potentiometric titration and chromatographic method as per IP, BP & USP[6-8]. Literature review reveals that HPLC[26-30] and extractive spectrophotometric[31-36] methods have been reported for estimation of ATN in pharmaceutical dosage forms.Literature survey does reveals only UV Spectrophotometric[37] and HPLC[37] methods have been developed and reported, but does not any extractive spectrophotometric method for simultaneous determination of LSKandATN in Pharmaceutical dosage form. The present developed method is simple, precise and accurate for simultaneous determination of both drugs in their Pharmaceutical Dosage forms as per International Conference on Harmonization (ICH) guidelines[9].

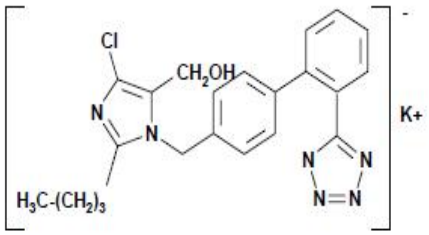

Fig. 1: Structure of Losartan potassium (LSK)

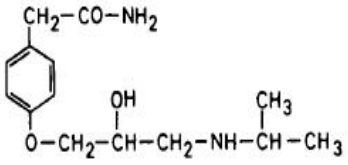

Fig.2:Structure of Atenolol (ATN)

EXPERIMENTAL:

INSTRUMENT: Absorbance measurements were made on Shimadzu UV-1700 double beam UV-Visible spectrophotometer with 10 mm matched quartz cells.

MATERIALS AND REAGENTS:

All materials and reagents used are of analytical grade; solvents are of spectroscopic grade and distilled water. Pure drug samples of Losartan potassium and Atenolol were provided as a gift sample by Olcare Laboratories, Surendranagar, Gujarat, India. Commercial pharmaceutical tablet **LOSAR*-BETA** (Unichem Laboratories, India) was procured from local pharmacy.

Preparation of standard stock solution of Losartan Potassium (1000µg/ml)

100 mg of Losartan potassium was weighed accurately and transferred to 100 ml volumetric flask. 50 ml of distilled water was added and allowed to shake for 5 minutes. Volume was made up to 100 ml with distilled water.

Preparation of standard stock solution of Atenolol (10000 µg/ml)

1000 mg of Atenololwas weighed accurately and transferred to 100 ml volumetric flask. 50 ml of distilled water was added and allowed to shake for 5 minutes. Volume was made up to 100 ml with distilled water.

Selection of solvent for extraction

Chloroform, Methylene chloride and benzene were tried for extraction. Chloroform was found to be the most convenient solvent for extraction. Other solvents were not suitable owing to the limited solubility of ion pair complex.

Selection of dye

Thenature of both drugs are polar; therefore many water soluble dyes were tried. All dyes were prepared as per the Pharmacopoeial and other official methods. Colour formation in chloroform layer was observed in three different pH medium with 1 ml of standard drug stock solutions and 1 ml of dye solution.

For the preparation of blank solutions, same procedure was followed without addition of drug solution in three different test tubes. If colour is observed in chloroform layer against the blank, it indicates that the ion pair complex has been formed which ischloroformextractable. About 20 dyes have been tried. Among all, following dye formed ion pair complex with respective drugs.

1. Ferroin solution with Losartan Potassium (Purple colour)
2. Methyl orange with Atenolol (Yellow colour)

Preparation of dye

1. Ferroin solution
   0.7 g of Ferrous sulphateand 1.5 g of 1,10-phenanthroline hydrochloride were weighed accurately and dissolvedin 70 ml of waterand sufficient water was addedto produce 100 ml.
2. Methyl orange
   0.1 g of Methyl orangewas weighed accurately and dissolved in 80 ml of waterand sufficientethanol (95 per cent)was addedto produce 100 ml.

Phosphate Buffer pH 7.0:

0.50 g of anhydrous disodium hydrogen phosphate and 0.301 g of potassium dihydrogen phosphate were weighed accurately and dissolvedin sufficient water to produce1000 ml.

Selection of analytical wavelength

The chloroform layers of selected dyes were collected in 10 ml volumetric flask and volume was made up to mark with chloroform. The resulting solutions containing 100µg/ml were scanned in UV-Visible spectrophotometer. The λ_{max} were found to be

Ferroin solution 571.0 nm
Methyl orange 426.0 nm

Optimization of reagents for both the drugs

A. For Losartan Potassium

- Volume of Phosphate Buffer pH 7.0:
  Solution of Phosphate Buffer pH 7.0was prepared and volume was added ranging from 0.5 ml to 5 ml to drug solution. Chloroform layer was collected after extracting and passing through anhydrous sodium sulphate bed and scanned for 300-800nm range. Peak absorbance was recorded and from the graph, it was found to be 3.0 ml of Phosphate Buffer pH 7.0(Figure 3, Table 1).

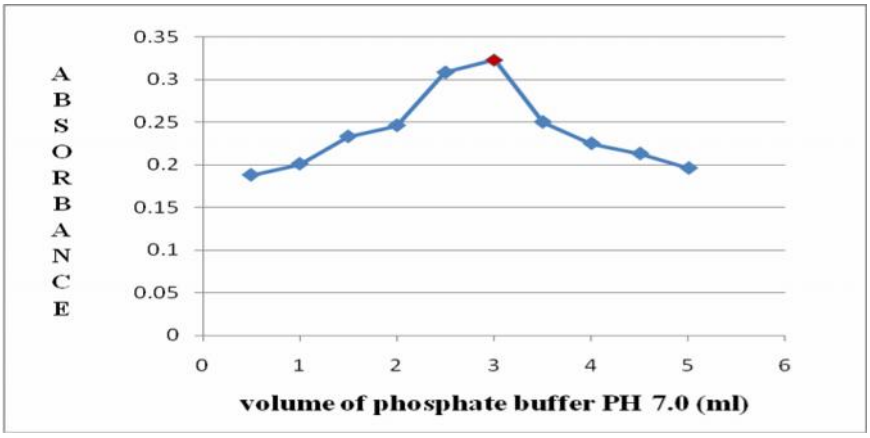

Fig. 3: Optimum Volume of Phosphate buffer PH 7.0 for Losartan Potassium

Table 1: Optimization of volume of Phosphate Buffer PH 7.0for Losartan Potassium

VOLUME (ml)	ABSORBANCE
0.5	0.188
1	0.201
1.5	0.233
2	0.246
2.5	0.308
3	**0.323**
3.5	0.25
4	0.225
4.5	0.213
5	0.196

- Volume of Ferroin solution :
  Volumes of dye ranging from 0.5 ml to 4.0 ml were added to Neutral drug solutions, Chloroform layer was collected after extracting and passing through anhydrous sodium sulphate bed and scanned for 300-800nm range. Peak absorbances were recorded, from the graph 1.5 ml of dye was found to be optimum (Figure 4, Table 2).

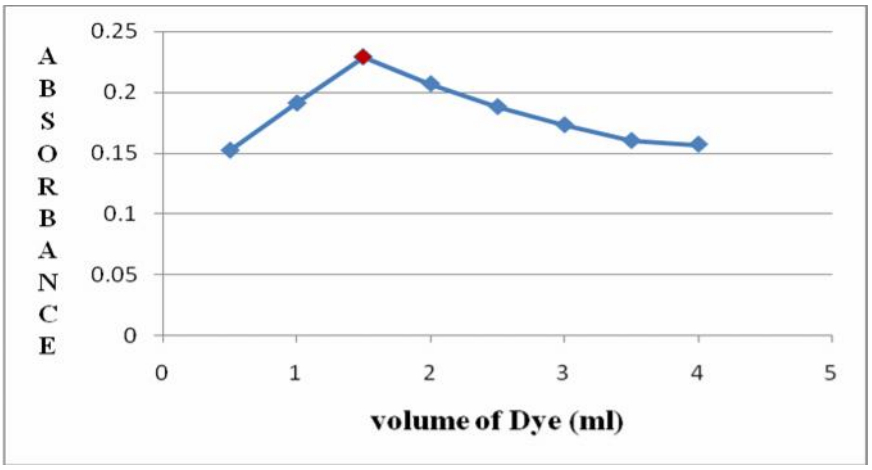

Fig. 4: Optimum Volume of Dye(Ferroin Solution) for Losartan Potassium

Table 2: Optimization of volume of dye (Ferroin Solution) For Losartan Potassium

VOLUME (ml)	ABSORBANCE
0.5	0.152
1	0.191
1.5	**0.229**
2	0.207
2.5	0.188
3	0.173
3.5	0.16
4	0.157

B. For Atenolol

- Volume of Phosphate Buffer pH 7.0:
  Solution of Phosphate Buffer pH 7.0was prepared and volume was added ranging from 0.5 ml to 3.5 ml to drug solution. Chloroform layer was collected after extracting and passing through anhydrous sodium sulphate bed and scanned for 300-800nm range. Peak absorbance was recorded and from the graph, it was found to be 2.0 ml of Phosphate Buffer pH 7.0(Figure 5, Table 3).

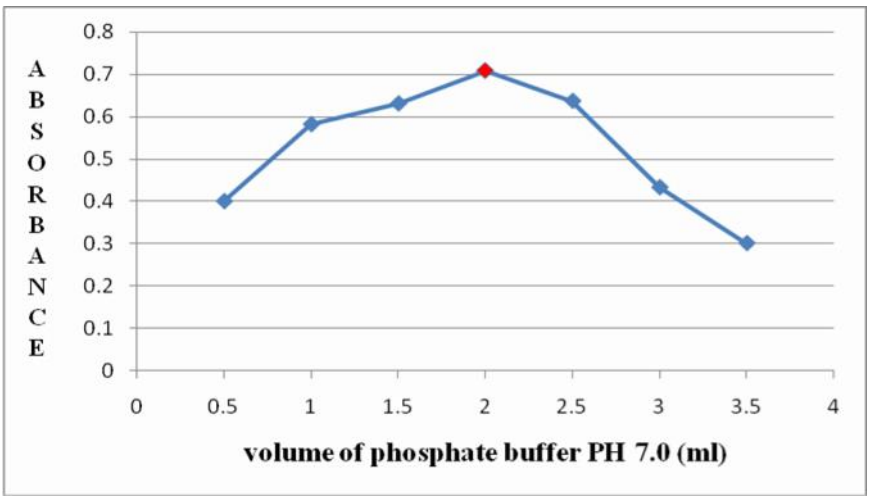

Fig. 5 : Optimum Volume of Phosphate buffer PH 7.0 for Atenolol

Table 3: Optimization of volume of Phosphate Buffer PH 7.0 for Atenolol

VOLUME (ml)	ABSORBANCE
0.5	0.4
1	0.582
1.5	0.631
2	**0.708**
2.5	0.637
3	0.433
3.5	0.301

- **Volume of Methyl Orange :**
  Volumes of dye ranging from 1.0 ml to 15.0 ml were added to Neutral drugsolutions, Chloroform layer was collected after extracting and passing through anhydrous sodium sulphate bed and scanned for 300-800nm range. Peak absorbances were recorded, from the graph 9.0 ml of dye was found to be optimum (Figure 6, Table 4).

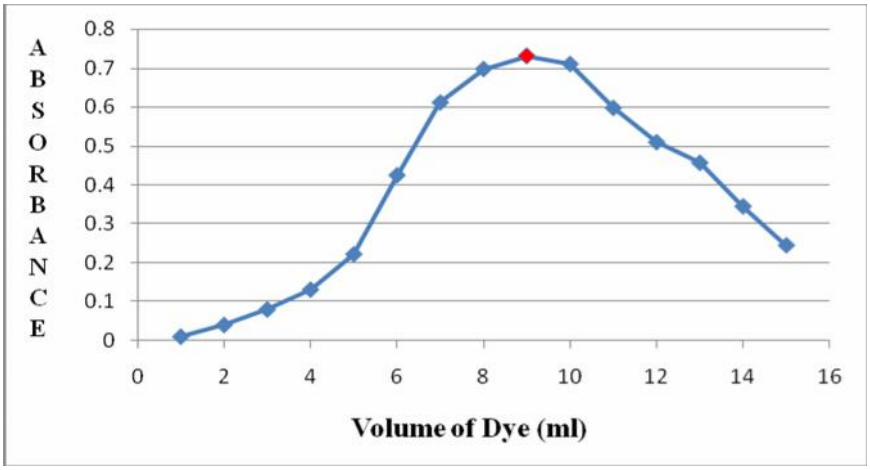

Fig. 6 : Optimum Volume of Dye (Methyl Orange) for Atenolol

Table 4: Optimization of volume of dye (Methyl Orange) For Atenolol

VOLUME (ml)	ABSORBANCE
1	0.01
2	0.04
3	0.08
4	0.131
5	0.222
6	0.425
7	0.612
8	0.698
9	**0.731**
10	0.711
11	0.598
12	0.51
13	0.457
14	0.345
15	0.245

Construction of Calibration curves:

A. For Losartan Potassium

In 125 ml separating funnel, appropriate aliquot (0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 ml) of Losartan Potassium from Standard stock solution was transferred, 1.5 mlof Ferroin Solution and 3.0 ml of Phosphate Buffer PH 7.0 were added in to it and mixed. The aqueous solution was extracted with an equal volume of chloroform (2 to 5 ml) and shaken for 45 sec and funnel was allowed to stand to get two phases separated. Organic layer was collected in dry 10 ml volumetric flask after passing through activated anhydrous sodium sulphate bed to remove aqueous traces. Volume was made up to 10 ml with chloroform and absorbance was measured at 571 nm(Figure 7, 9& Table 5).

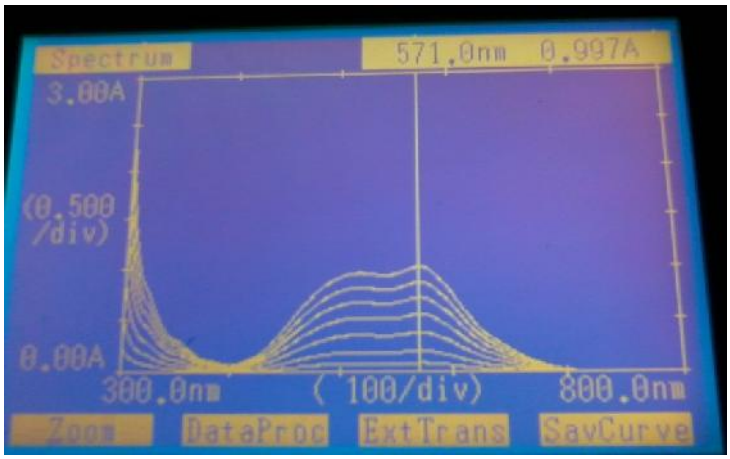

Fig.7: Overlay Spectrum of Losartan Potassium 25µg/ml-300 µg/ml at 571.0 nm

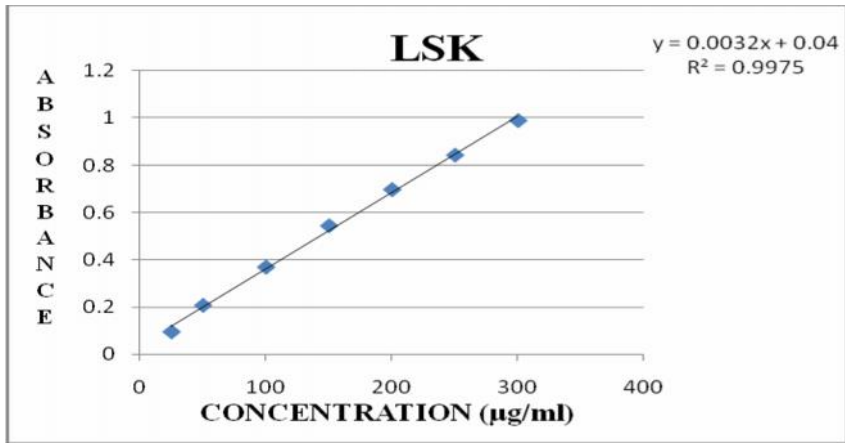

Fig. 9 : Calibration curve for Losartan Potassium

Table 5: Calibration Curve Datafor Losartan Potassium at 571.0 nm

CONCENTRATION (µg/ml)	MEAN ± S.D.(n=6)	% RSD
25	0.095 ± 0.0019	1.9797
50	0.207 ± 0.0019	0.906
100	0.369 ± 0.0015	0.3987
150	0.544 ± 0.0023	0.4257
200	0.697 ± 0.0015	0.2112
250	0.843 ± 0.0018	0.2176
300	0.989 ± 0.0019	0.1893

B. For Atenolol

In 125 ml separating funnel, appropriate aliquot (0.05, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 ml) of Atenolol from Standard stock solution was transfered, 9.0 ml of Methyl orange and 2.0 ml of phosphate Buffer PH 7.0 were added in it and mixed. The aqueous solution was extracted with an equal volume of chloroform (2 to 5 ml) and shake for 45 sec and flask was allowed to stand to get two phase separated. Organic layer was collected in dry 10 ml volumetric flask after passing through activated anhydrous sodium sulphate bed to remove aqueous traces. Volume was made up to 10 ml with chloroform and absorbance was measured at 426.0 nm(Figure 8,10& Table 6).

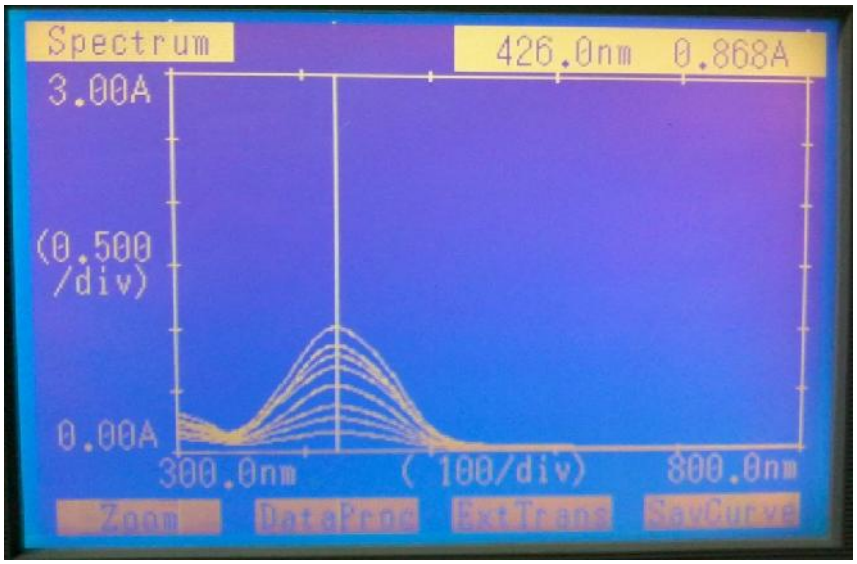

Fig. 8 : Overlay Spectrum of Atenolol 50 µg/ml-1400 µg/ml at 426.0 nm

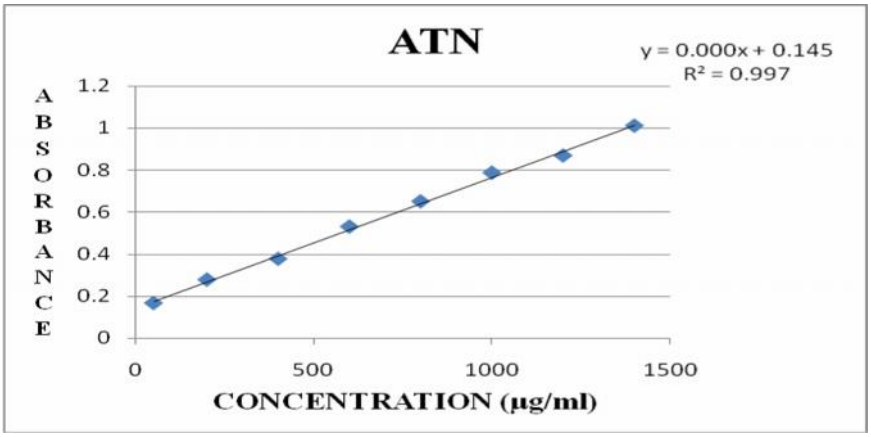

Fig. 10 : Calibration curve for Atenolol

Table 6: Calibration Curve Data for Atenolol at 426.0 nm

CONCENTRATION (µg/ml)	MEAN ± S.D. (n=6)	% RSD
50	0.167 ± 0.0022	1.2962
200	0.278 ± 0.0021	0.7682
400	0.377 ± 0.0023	0.6148
600	0.530 ± 0.0023	0.4429
800	0.650 ± 0.0021	0.3179
1000	0.786 ± 0.0025	0.3186
1200	0.867 ± 0.0021	0.2382
1400	1.009 ± 0.0019	0.1855

Assay of Drugs from pharmaceutical dosage form

Twenty tablets were weighed and powdered in a glass mortar. Tablet powder equivalent to 100 mg of Losartan Potassium and Atenolol were weighed accurately and transferred into 100 ml volumetric flask. The drug was dissolved in distilled water and the volume was made up to the mark. The solution was suitably diluted and assayed as under the respective assay procedure described for the preparation of calibration curves for both the drugs. Results are listed in Table 7.

Table 7: Assay Results of Tablet dosage form

FORMULATION	ACTUAL CONC. OF DRUG (µg/ml)		AMT. OF DRUG FOUND (µg/ml)		% OF DRUG FOUND		%MEAN		% RSD	
	LSK	ATN	LSK	ATN	LSK	ATN	LSK	ATN	LSK	ATN
LOSAR -BETA	100	200	96.87	200.16	96.87	100.08	96.04	100.08	0.8179	0.8343
	100	200	95.31	198.5	95.31	99.25				
	100	200	95.94	201.8	95.94	100.92				

Method Validation [10, 11]

1. **Linearity and Range**: The linearity was determined at six levels over the range of 25-300 µg/ml and 50-1400 µg/ml for Losartan Potassium and Atenolol respectively. Absorbances of above linearity solution preparations were taken at each concentration six times. Mean absorbance at each concentration was calculated and Graph of mean absorbance (y-axis) versus concentration (x-axis) was Plotted. Values of correlation co-efficient (r), y-intercept & slope of regression line were Calculated and Recorded (Figure 7, 8, 9, 10 & Table 5, 6).
2. **Accuracy:** Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy 20 Tablets were taken and weighed. Powder equivalent to 100 mg was taken and analysis of the same was carried out. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80%, 100% & 120%) taking into consideration percentage purity of added bulk drug samples (Table 8, 9).
3. **Precision:** The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It provides an indication of random errors in results and was expressed as % RSD (Table 10, 11).

a) **Repeatability**
Standard solutions of Losartan Potassium (25-300 µg/ml) and Atenolol (50-1400 µg/ml) were prepared and spectrums were recorded. Absorbances were measured at 571.0 and 426.0 nm for Losartan Potassium and Atenolol respectively using chloroform as a blank. The absorbances of the same concentration solution were measured six times and % RSD was calculated.

b) **Intra and inter-day precision**:Variation of results within the same day (intra- day) and variation of results between days (inter- day) were analyzed. Intra-day precision was determined by analysing Losartan Potassium and Atenololfor three times in the same day. Inter-day precision was determined by analysing Losartan Potassium and Atenololdaily for three days.

4. **Specificity and Selectivity:** Specificity is a procedure to detect quantitatively the analyte in presence of all degraded product & components that may be expected to be present in the sample matrix. While selectivity is the procedure to detect qualitatively the analyte & all degradation product in presence of components that may be expected to be present in the sample matrix.
5. **Solution Stability study / stability of colour:** Sample solution was prepared as per test procedure. The Absorbance of Sample Solution after extraction was monitored by UV-Visible spectrophotometer system at regular intervals for 24 hrs. Data were recorded which indicates required colour stability(Table 12).
6. **Limit of Detection and Limits of Quantitation:** From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. The limit of detection (LOD) of the drug was calculated by using the following equation designated by International Conference on Harmonization (ICH) guideline:

$$LOD = 3.3\pm / S$$

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

From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. The limit of quantitation (LOQ) of the drug was calculated by using the following equation designated by International Conference on Harmonization (ICH) guideline:

$$LOQ = 10\pm / S$$

Where, σ = the standard deviation of the response
S = slope of the calibration curve

Table 8: Determination of Accuracyfor Losartan Potassium

AMT. OF SAMPLE (mg)	AMT.OF DRUG ADDED(mg)	AMT. OF DRUG RECOVERED (mg)	%RECOVERY	MEAN	SD	%RSD
100	80	79.72	99.31			
100	80	79.44	99.65			
100	80	80.14	100.17	99.71	0.433128	0.434388
100	100	200.31	100.16			
100	100	199.38	99.68			
100	100	200.63	100.31	100.05	0.32909	0.328925
100	120	218.75	119.32			
100	120	219.69	119.83			
100	120	220.63	120.34	119.83	0.51	0.425603

Table 9: Determination of Accuracy for Atenolol

AMT. OF SAMPLE (mg)	AMT. OF DRUG ADDED (mg)	AMT. OF DRUG RECOVERED (mg)	%RECOVERY	%MEAN	SD	%RSD
200	160	159.33	99.58			
200	160	158.59	99.12	99.58	0.46	0.46194
200	160	160.07	100.04			
200	200	200.08	100.04			
200	200	200.92	100.45	100.0367	0.41501	0.414858
200	200	199.25	99.62			
200	240	239.18	99.65			
200	240	238.27	100.04	99.65667	0.380044	0.381353
200	240	240.09	99.28			

Table 10 : Intra-Day and Inter-Day Precision study of Losartan Potassium

Concentration (µg/ml)	Intra-Day Absorbance Mean (n=3) ± SD	%RSD	Inter-Day Absorbance Mean (n=3) ± SD	%RSD
100	0.369 ± 0.0015	0.4136	0.369 ± 0.0020	0.5420
150	0.545 ± 0.0015	0.2805	0.544 ± 0.0030	0.5515
200	0.697 ± 0.0015	0.2193	0.695 ± 0.0015	0.2199

Table 11 : Intra-Day and Inter-Day Precision study of Atenolol

Concentration (µg/ml)	Intra-Day Absorbance Mean (n=3) ± SD	%RSD	Inter-Day Absorbance Mean (n=3) ± SD	%RSD
400	0.376 ± 0.0015	0.4066	0.378 ± 0.0031	0.8075
600	0.531 ± 0.0025	0.4742	0.534 ± 0.0031	0.5717
800	0.650 ± 0.0015	0.2349	0.654± 0.0040	0.6183

Table 12: Colour stability monitoring

TIME	ABSORBANCE	
	LSK (100 µg/ml)	ATN (200 µg/ml)
10.00am	0.369	0.278
1.00 pm	0.368	0.278
6.00pm	0.366	0.276
10.00am	0.364	0.273

Table 13: Optical characteristics of proposed method for the Drugs

Parameter	LOSARTAN POTASSIUM	ATENOLOL
λ max (nm)	571	426
Linear Range (µg/ml)	25-300	50-1400
Regression Equation* (y)	y=bx+a	y=bx+a
Slope (b)	0.0032	0.0006
Intercept (a)	0.0400	0.1459
Correlation coefficient(r^2)	0.9975	0.9974
Standard deviation of slope	0.0000	0.0000
Standard deviation of intercept	0.0018	0.0022
Limit of Detection (µg/ml)	1.8717	12.0065
Limit of Quantitation (µg/ml)	5.6719	36.3833

**Y = bx+a, where 'Y' is the absorbance and x is the concentration of drugs (µg/ml) For six replicates.*

RESULTS AND DISCUSSION:

The proposed method is simple, accurate and precise. Losartan Potassium and Atenolol were found to yield a clear Purple and Yellow colored complexes with Ferroin Solution and Methyl orange respectively which were extractable with chloroform having the absorption maxima of 571.0 and 426.0 nm respectively. The colored products were due to the ion pair complex formation of the drug with the dye in the presence of Phosphate buffer of pH 7.0. The linearity ranges were found to be 25-300 µg/ml and 50-1400 µg/ml for Losartan Potassium and Atenolol respectively.Validation parameters have been performed such as Limit of detection (LOD), Limit of quantitation (LOQ), accuracy, precision, robustness, specificity and selectivity as per International conference of Harmonization (ICH) guidelines[9]. The optical characteristics such as Beer's law limit, the regression analysis using the method of least square was made for the slope (b), intercept (a). Correlation coefficient (r) were also determined for the proposed method and results are presented in Table-13. Commercial formulation containing both drugs was successfully analyzed by the reference and proposed method. The Recovery studies were performed by adding a fixed amount of the drug to the pre analysed formulation. Interference studies reveals that the common excipients and other additives usually present in the dosage form did not interfere in the proposed method.

CONCLUSION:

The method was successfully validated and applied to marketed formulation and excellent results were obtained with both the dyes. Extractive Spectrophotometric method was found to be simple, accurate and precise. As the method is selective and specific, it can be useful to the routine analysis of drugs.

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