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Development and Validation of Stability Indicating Analytical Method for Simultaneous Estimation of Perindopril and Losartan Potassium in Their Combined Marketed Dosage Form

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ABSTRACT:

A simple, precise and accurate stability indicating RP-HPLC method was developed and validated for simultaneous estimation of Perindopril and Losartan Potassium in their combined marketed dosage form. Validation parameter proves that method is repeatable, sensitive and selective for the analysis of Perindopril and Losartan Potassium. Based on this evidence the method can be stated as highly economical and it is recommended for routine analysis and stability studies. In this method Buffer (Potassium Dihydrogen Phosphate): Methanol (80:20v/v) was used as a mobile phase and C18 (25cm x 0.46 cm) Hypersil BDS analytical column was used for the separation of drug with other degraded product. The flow rate 1.0 ml/min, detection wavelength 230 nm was used. The retention time for Perindopril and Losartan Potassium was found to be 3.867 minute and 5.287 minute. The linearity for Perindopril and Losartan Potassium was obtained in the concentration range of 2-6 µg/ml and 50-150 µg/ml with accuracy of 99.59-99.88% and 99.63-99.92 %. The developed method meets all the acceptance criteria for the validation of analytical method as per the ICH guideline. The degradation of Perindopril and Losartan Potassium in acid, base, oxidation, thermal and Photolytic condition was found to be 23.09%, 20.27%, 24.86%, 22.06% and 21.00% and for Losartan Potassium 21.17%, 23.00%, 25.77%, 21.65% and 19.74% respectively.

Key words: Perindopril, Losartan Potassium, Stability indicating RP-HPLC, validation.

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INTRODUCTION:

Perindopril is a nonsulfhydryl prodrug that belongs to the angiotensin converting enzyme (ACE) inhibitor class of medications. Perindopril may be used to treat mild to moderate essential hypertension, mild to moderate congestive heart failure, and to reduce the cardiovascular risk of individuals with hypertension or post-myocardial infarction and stable

coronary disease. Perindopril may be used to treat mild to moderate essential hypertension, mild to moderate congestive heart failure, and to reduce the cardiovascular risk of individuals with hypertension or post-myocardial infarction and stable coronary disease. Losartan is an angiotensin-receptor blocker (ARB) that may be used alone or with other agents to treat hypertension. Losartan may be used to treat isolated systolic hypertension, left ventricular hypertrophy and diabetic nephropathy. It may also be used as an alternative agent for the treatment of systolic dysfunction, myocardial infarction, coronary artery disease, and heart failure. Losartan competitively inhibits the binding of angiotensin II to AT1 in many tissues

aldosteronesecreting effects and results in decreased including vascular smooth muscle and the adrenal glands. Inhibition of angiotensin II binding to AT1 inhibits its AT1-mediated vasoconstrictive and vascular resistance and blood pressure.

MATERIALS AND METHODS:

Apparatus:

Model: TSP, Column: C18 (25 cm × 0.46 cm) Hypersil BDS, Injector: 20µL fixed loop, Detector: UV Detector, Software: LC, Analytical balance: Electronic analytical balance (Shimadzu).

Reagents and Materials:

Perindopril was procured from Torrent Pharma, Losartan was procured from Torrent Pharma, Methanol, Potassium Dihydrogen Phosphate

Chromatographic Conditions:

Column: C18 (25cm x 0.46 cm) Hypersil BDS, Mobile Phase: Buffer (Potassium Dihydrogen Phosphate, pH 5.0): Methanol (80:20), Flow Rate: 1.0 ml/min, Detection Wavelength: 230nm, Run time: 8 min, Injection volume: 20.0 µl.

Preparation of Standard Solutions:

A) Perindopril standard stock solution: (40 µg/mL)

A 4 mg of Perindopril was weighed and transferred to a 100 mL volumetric flask. Volume was made up to the mark with methanol.

B) Losartan standard stock solution: (1000 µg/mL)

A 100 mg of Losartan was weighed and transferred to a 100 mL volumetric flask.

Volume was made up to the mark with methanol.

C) Preparation of standard solution of mixtures of Perindopril(4µg/mL) and Losartan (100µg/mL)

Take 1 mL from the Perindopril stock solution and 1mL from Losartan stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase which was used in particular trials.

Preparation of Mobile Phase

Mobile phases were prepared by mixture of Buffer solution (Potassium Dihydrogen phosphate, pH 5.0): Methanol (80:20)

Selection of Detection wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study drug solutions of Perindopril (4 ppm) and Losartan (100 ppm) were prepared in Methanol. These drug solutions were than scanned in UV region of 200-400 nm and overlay spectrums were recorded.

Preparation of Calibration Curves

The linearity for Perindopril and Losartan were assessed by analysis of combined standard solution in range of 2-6µg/ml and 50-150µg/ml respectively, 0.5,0.75,1,1.25,1.5 ml solutions were pipette out from the Stock solution of Perindopril (40µg/ml) and Losartan (1000µg/ml) and transfer to 100 ml volumetric flask and make up with mobile phase to obtain 2,3,4,5 and 6µg/ml, and 50,75,100,125 and 150µg/ml for Perindopril and Losartan respectively. Mean area AUC against concentration were plotted to obtain the calibration curve. Regression equation, co-relation coefficients were computed calibration curves.

Estimation of Perindopril and Losartan Potassium in marketed tablet

Weigh 20 tablets accurately and powdered. A tablet powder equivalent to 4 mg of Perindopril and 100 mg of Losartan was taken into 100 ml volumetric flask. Add sufficient volume of mobile phase to dissolve and sonicate for 25 min. make up the volume up to mark with mobile phase and filter through whatman filter paper. From this above solution, suitable aliquots were transferred into 10 ml volumetric flask and volume was made up to the mark with mobile phase to get final concentration of 40µL and 100µL for Perindopril and losartan respectively.

METHOD VALIDATION

As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantification.

Linearity and Range

The linearity for Perindopril and Losartan were assessed by analysis of combined standard solution in range of 2-6µg/ml and 50-150µg/ml respectively. Correlation co-efficient for calibration curve Perindopril and Losartan Potassium was found to be 0.995 and 0.996 respectively. The regression line equation for Perindopril: **y = 558.5x – 3.266**, and for Losartan: **y = 23.21x – 3.023**

Accuracy

For Perindopril:

2 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 230 nm. The amount of Perindopril was calculated at each level and % recoveries were computed.

For Losartan:

50 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 230 nm. The amount of Losartan was calculated at each level and % recoveries were computed.

Precision

Results should be expressed as Relative standard deviation (RSD) or coefficient of variance.

A. **Repeatability:** Standard solution containing Perindopril (4 µg/ml) and Loartan (100 µg/ml) was injected six times and areas of peaks were measured and % R.S.D. was calculated.
B. **Intra-day precision:** Standard solution containing (2,4,6 µg/ml) of Perindopril and (50,100,150 µg/ml) of Losartan were analyzed three times on the same day and % R.S.D was calculated.
C. **Inter-day precision:** Standard solution containing (2,4,6 µg/ml) of Perindopril and (50,100,150 µg/ml) of Losartan were analyzed three times on the different day and % R.S.D was calculated.

Limit of Detection and Limit of Quantification

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD = 3.3 * SD/slope of calibration curve

LOQ = 10 * SD/slope of calibration curve

Where, SD = Standard deviation of intercepts

RESULTS AND DISCUSSION

Linearity study was carried out at five different concentration levels. The linearity for Perindopril and Losartan were assessed by analysis of combined standard solution in range of 2-6µg/ml and 50-150µg/ml.

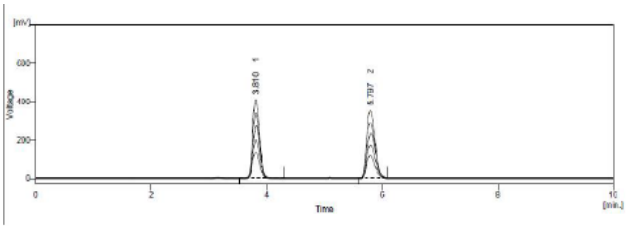

Figure 1: Overlaid Chromatogram of Perindopril and Losartan

Table: 1 Linearity data for Perindopril at 230nm

Concentration (µg/ml)	Absorbance at 230 nm Mean ± S.D. (n=5)
2	1124.944 ± 1.282
3	1661.123 ± 0.928
4	2271.304 ± 1.121
5	2696.764 ± 1.270
6	3399.654 ± 0.971

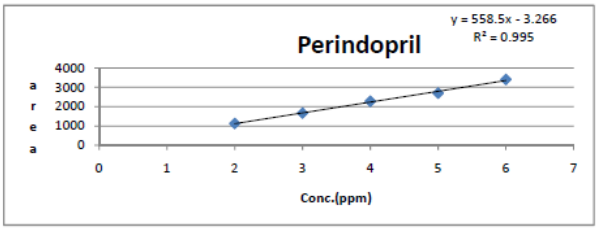

Figure 2: Calibration Curve of Perindopril (2-6µg/ml)

Table: 2 Linearity data for Losartan at 230nm

Concentration (µg/ml)	Absorbance at 230 nm Mean ± S.D. (n=5)
50	1169.117 ± 1.064
75	1725.923 ± 1.118
100	2359.509 ± 1.160
125	2805.077 ± 1.085
150	3530.968 ± 1.126

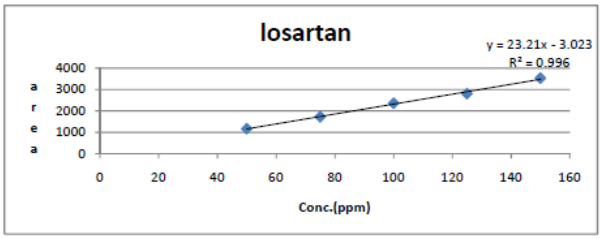

Figure 3: Calibration Curve of Losartan (50-150µg/ml)

Table: 3 Repeatability

Drugs	Conc. (µg/ml)	Area (mAU*S) Mean ± S.D. (n=6)	%RSD
Per	4	2263.877 ± 12.52	0.553
Los	100	2349.318 ± 16.509	0.702

Table: 4 Intraday Precision

Drugs	Conc. (µg/ml)	Mean Area (mAU*S) ± S.D. (n=3)	%RSD
Per	2	1116.215 ± 8.44	0.756
	4	2249.788 ± 23.766	1.056
	6	3376.40 ± 0.061	0.601
Los	50	1160.443 ± 6.602	0.568
	100	2339.543 ± 17.24	0.737
	150	3508.760 ± 14.092	0.401

Table: 5 Interday Precision

Drugs	Conc. (µg/ml)	Mean Area (mAU*S) ± S.D. (n=3)	%RSD
Per	2	1109.399 ± 15.254	1.374
	4	2249.03 ± 14.973	0.665
	6	3372.257 ± 9.346	0.277
Los	50	1159.537 ± 6.613	0.570
	100	2344.514 ± 6.103	0.260
	150	3493.61 ± 0.986	0.986

Table: 6 Accuracy study for Perindopril and Losartan

Drug	Level	Amount of API added	Mean% Recovery	SD	%RSD
Per	80	1.6	99.881	0.965	0.966
	100	2	99.620	0.489	0.491
	120	2.4	99.594	0.413	0.415
Los	80	40	99.924	1.127	1.127
	100	50	99.633	0.649	0.652
	120	60	99.632	0.490	0.492

Table: 7 System suitability parameters

Parameters	For Perindopril	For Losartan	Standard Value
Retention Time (R_t)	3.867	5.287	-
Theoretical plates (N)	4435	7198	Should be > 2000
Tailing Factor	1.323	1.394	0.9 – 2.0
Resolution	5.898		-

Table:8 Assay of Formulation

Formulation	Perindopril			Losartan		
Adpace (2mg)	**Amount labelled (mg)**	**Amount Found (mg)**	**% amount found ± SD (n=3)**	**Amount labelled (mg)**	**Amount Found (mg)**	**% amount found ± SD (n=3)**
	2	1.931	96.571 ± 0.622	50	47.815	95.631 ± 0.369

CONCLUSION

A simple, selective, precise and accurate stability indicating RP-HPLC method for analysis of Perindopril and Losartan Potassium in combined marketed dosage form was developed and validated. The chromatographic conditions comprised of a reversed phase c18 column,

with a mobile phase composed of mixture of Potassium Dihydrogen phosphate buffer at pH 5.0 adjusted with Methanol in ratio of 80:20. Flow rate was adjusted to 1 ml/ min. Detection was carried out at 230 nm. The retention of Perindopril was 3.8 min and 5.2 min for Losartan. The drug undergoes degradation under thermal, acidic, basic and peroxide & photolytic condition. Peak of degraded product was resolved from the active pharmaceutical ingredient.

A developed and validated method was found to be simple, accurate, rapid, economical and reliable. Obtained results comply with all the system suitability requirements along with peak purity of both components in all stressed conditions. The study indicates that there is non-interference of any degradant with the analyte peak, which proves that the method is specific for estimation of Perindopril and Losartan in the formulation.

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