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Research Article

A New Stability Indicating RP-UFLC Method for the Estimation of Perindopril Erbumine Tablet Dosage Forms

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Abstract

Perindopril erbumine is used for the treatment of heart failure, hypertension and other coronary artery diseases. A new stability indicating RP-UFLC method has been proposed for the estimation of Perindopril erbumine in tablet dosage forms. A mixture of 10 mM Tetra butyl ammonium hydrogen sulphate (pH 3.4): Acetonitrile: 0.1% Acetic acid (48:52: 0.1) was chosen as mobile phase (Isocratic mode) with flow rate 0.8 mL/min (Detection wavelength 210 nm). Shimadzu Model UFLC system with PDA detector and Luna C8 Column was used for the study**.** Perindopril erbumine obeys Beer-Lambert's law over the concentration range 0.2-120 µg/mL with linear regression equation y = 47597x – 10133 (R^2 = 0.9999). The LOQ and LOD were found to be 0.1981 µg/mL and 0.0641 µg/mL. Stress degradation studies were performed such as acid hydrolysis, alkaline hydrolysis, thermal treatment and oxidation according to the ICH guidelines. The proposed UFLC method has validated and found to be simple, precise, accurate, robust and is very much useful for the analysis of pharmaceutical formulations (Tablets).

Keywords: Perindopril Erbumine; RP-UFLC; Stability Indicating; Validaion; ICH Guidelines

Introduction

Perindopril erbumine (CAS no. 10713336) is a long-acting ACE inhibitor. It is chemically (2S,3aS,7aS)-1-[(2S)-2-[[(2S)-1-ethoxy-1-oxo pentan-2-yl] amino] propanoyl]-2, 3, 3a, 4, 5, 6, 7, 7a-octa hydro indole-2-carboxylic acid; 2-methyl propan-2-amine (Figure 1). Perindopril erbumine ($C_{19}H_{32}N_2O_5C_4H_{11}N$) is the tertiary butyl amine salt of Perindopril. It is commonly used for the treatment of high blood pressure, heart failure, hypertension and other coronary artery diseases [1,2]. Perindopril erbumine converts in to Perindoprilat during metabolism and inhibits angiotensin converting enzyme which results in the conversion of angiotensin I to angiotensin II which results in diuresis and anti-hypertensive effect.

Figure 1: Chemical structure of Perindopril erbumine.

Hari Hara Theja., *et al*. developed a stability-indicating RP-HPLC method [3] for the quantitative analysis of Perindopril erbumine in tablet dosage form using Phenomenex Luna C18 column with mobile phase 0.2% TFA (pH adjusted to 3.0 with ammonia): acetonitrile (60:40) (Detection wavelength 215 nm). Linearity was observed as 2.5-50 µg/mL and four major impurities were observed with retention time 2.7 min (Impurity 1), 2.9 min (Impurity 2), 3.2 min (Impurity 3) and 6.7 min (Impurity 4) with resolution more than 2.

Medenica., *et al.* evaluated [4] the impurities of Perindopril tert-butylamine using a Hewlett Packard 1100 chromatographic system with YMC-Pack C8 column. Mobile phase mixture consisting of acetonitrile and 0.05 M potassium phosphate buffer (pH adjusted to 2.5 with ortho phosphoric acid) (37:63) was used with flow rate 1.7 ml/min (Detection wavelength 215 nm) and mixture of acetonitrile: water (40:60) was used as diluent. The linearity of Perindopril tert-butylamine, Peridoprilat and other three impurities were found to be 0.01-1.0 mg/ml, 0.2-20, 0.2-20 and 0.1-10 µg/ml respectively. Prameela Rani., *et al*. developed a RP-HPLC method [5] for the determination of Perindopril in pharmaceutical formulations using C 18 column and mobile phase consisting of methanol: water (4:1) i. e 80: 20 v/v with flow rate 1 ml/min (Detection wavelength 215 nm) and linearity was observed over the concentration range 4-20 µg/ml. Yasmeen., *et al*. developed a RP-HPLC method [6] for the determination of Perindopril erbumine in tablet dosage forms using Symmetry C18 Phenomenex column and mobile phase consisting of phosphate buffer (pH was adjusted to 5 by using ortho phosphoric acid): methanol (30:70) with flow rate 0.8 ml/min (Detection wavelength 215 nm) where Perindopril erbumine was eluted at about 2.45 min and the linearity was observed over the concentration range 10-50 µg/ml. Lin., *et al*. developed a capillary gas chromatographic method [7] for the determination of Perindopril which is based on the derivatization of Perindopril with penta fluoro benzyl bromide in acetone, using potassium carbonate as a base catalyst. The resulting derivative was separated by using SE-54 capillary column and flame-ionization detector and the linearity was found to be Perindoplil was over 20-300 nmol. In the present study a new RP-HPLC method has been developed for the estimation of Perindopril and the method was validated as per ICH guidelines.

Materials and Methods

Instrumentation and Chromatographic conditions

Shimadzu Model UFLC system with PDA detector and Luna C8 Column was used for the present study. A mixture of 10 mM Tetra butyl ammonium hydrogen sulphate (pH 3.4): Acetonitrile: 0.1% Acetic acid (48:52: 0.1) was chosen as mobile phase (Isocratic mode) with flow rate 0.8 mL/min (Detection wavelength 210 nm).

Preparation of Perindopril erbumine solution

25 mg of Perindopril erbumine (API) was weighed accurately and transferred into a 25 mL volumetric flask and dissolved in HPLC grade acetonitrile (1000 µg/mL) (Stock solution). and sonicated for 30 mins and dilutions were made with the mobile phase. All the solutions were filtered before use through membrane filter.

Method validation [8]

Linearity, precision, accuracy and robustness

A series of solutions of Perindopril erbumine (0.2-120 µg/mL) were prepared from the stock solution in 10 ml volumetric flasks with mobile phase, sonicated and filtered through membrane filter. 20 µl of each of these filtered solutions were injected (n = 3) into the UFLC system and the peak area of each chromatogram was noted. Then the mean peak area was calculated and a calibration graph was drawn by plotting the concentration of the drug solutions on the x-axis and the corresponding mean peak area of the chromatograms on the y-axis.

The intraday precision studies were performed on the same day at different equal intervals of time by injecting six solutions (10 µg/mL) and the interday precision studies were conducted by injecting the drug solution (10 µg/mL) on three successive days (Day 1, Day 2 and Day 3) and the % RSD was calculated.

Accuracy studies were performed by spiking the formulation solution with 50, 100 and 150% of API of Perindopril erbumine and the resulting solutions were made up to volume in the volumetric flaks with the mobile phase and injected in to the UFLC system. The peak area of the chromatograms was noted and thereby the mean peak area, % RSD and the percentage recovery were calculated from the calibration curve.

In robustness study, small changes such as mobile phase ratio, pH, flow rate, detection wavelength etc. were incorporated

purposefully in the optimised chromatographic conditions and the method was studied.

Stress degradation studies [9]

Stress degradation studies were performed for studying the specificity of the proposed method. Perindopril erbumine was allowed to undergo acidic hydrolysis, alkaline hydrolysis, oxidation and thermal degradation reactions.

Acidic hydrolysis was performed by treating Perindopril erbumine solution with 1 mL of 0.1 N HCl solution for 60 minutes at room temperature. The stressed sample was then neutralized with 1.0 mL 0.1N sodium hydroxide solution and made up to volume with the mobile phase. 20 µl of the resulting mixture was injected in to the UFLC system and the peak area of the chromatogram of Perindopril erbumine was noted and the percentage degradation was calculated.

Alkaline hydrolysis was performed by treating Perindopril erbumine solution with 1.0 mL 0.1N sodium hydroxide solution for 60 minutes at room temperature. The stressed sample was then neutralized with 1 mL of 0.1 N HCl solution and made up to volume with the mobile phase. 20 µl of the resulting mixture was injected in to the UFLC system and the peak area of the chromatogram of Perindopril erbumine was noted and the percentage degradation was calculated.

Oxidation reaction was performed by treating Perindopril erbumine solution with 1 mL 30% H_2O_2 solution for 60 minutes at room temperature and made up to volume with the mobile phase. Then 20 µl of the stressed sample was injected in to the UFLC system and the peak area of the chromatogram of Perindopril erbumine was noted and the percentage degradation was calculated.

Thermal degradation was performed by heating Perindopril erbumine solution at 40ºC for 60 minutes in water bath and made up to volume with the mobile phase. Then 20 µl of the stressed sample was injected in to the UFLC system and the peak area of the chromatogram of Perindopril erbumine was noted and the percentage degradation was calculated.

Assay of perindopril erbumine tablets

Perindopril erbumine is available with brand names Coversyl (Serdia Pharmaceuticals India Pvt. Ltd; Label claim: 2 mg, 4 mg, 8 mg), Perigard (Glenmark Pharmaceuticals) (Label claim: 4 mg) and Perindprim (Steris Pharma; (Label claim: 4 mg) in India.

20 tablets of two different brands were collected from the local pharmacy store, weighed and tablet powder equivalent to 25 mg Perindopril erbumine was transferred to two different 25 ml volumetric flasks and acetonitrile was added. The mixture was sonicated thoroughly and filtered through membrane filter and dilutions were made using the mobile phase as per requirement. 20 µL of each of these solutions were injected (n=3) in to the UFLC system and the percentage recovery was calculated from the peak area of the chromatogram ad the calibration curve.

Results and Discussion

A new stability indicating RP-UFLC method has been proposed for the estimation of Perindopril erbumine in tablet dosage forms. Shimadzu Model UFLC system with PDA detector and Luna C8 Column was used for the study. A review of the literature was thoroughly discussed earlier and some of the parameters were highlighted in table 1. A mixture of 10 mM Tetra butyl ammonium hydrogen sulphate (pH 3.4): Acetonitrile: 0.1% Acetic acid (48:52: 0.1) was chosen as mobile phase (Isocratic mode) with flow rate 0.8 mL/min (Detection wavelength 210 nm) for the quantification of Perindopril erbumine. A typical chromatogram of placebo and that of Perindopril erbumine was shown in figure 2.

Table 1: Literature survey.

Mobile phase (v/v)	Column	λ (nm)	Linearity (µg/mL)	Comment	Ref
0.2% TFA (pH adjusted to 3.0 with ammonia): acetonitrile (60:40)	Phenomenex Luna C18	215	2.5-50	RP-HPLC	[3]
Acetonitrile:0.05M Potassium phosphate buffer (pH adjusted to 2.5 with ortho phosphoric acid) (37:63)	YMC-Pack C8	215	10-1000	RP-HPLC (Impurities)	[4]

Methanol: Water (80:20)	C18	215	4-20	RP-HPLC	[5]
Phosphate buffer (pH was adjusted to 5 by using ortho phosphoric acid): methanol (30:70)	Symmetry C18 Phenomenex	215	10-50	RP-HPLC	[6]
Derivatization with penta fluoro benzyl bromide in acetone using Potassium carbonate as a base catalyst	SE-54 capillary column	-	20-300 nmol	Capillary Gas chromatography	[7]
10 mM Tetra butyl ammonium hydrogen sulphate (pH 3.45): Acetonitrile: 0.1% Acetic acid (48:52: 0.1)	Luna C8 column	210	0.2-120	RP-UFLC	Present work

Linearity, precision, accuracy and robustness

Perindopril erbumine obeys Beer-Lambert's law over the concentration range 0.2-120 µg/mL (% RSD 0.22-0.84) (Table 2) with linear regression equation y = 47597x – 10133 (R^2 = 0.9999) (Figure 3). The LOQ and LOD were found to be 0.1981 µg/mL and 0.0641 µg/mL. The % RSD in precision study was found to be 0.0224 (Intraday) (Table 3) and 0.1603 (Interday) (Table 4) indicating that the method is precise. The % RSD in accuracy study was found to be 0.35-0.89 (Table 5) (and that of robustness study was 0.22-1.02 (Table 6) indicating that the method is accurate (% Recovery 99.35-99.72) and robust.

Table 2: Linearity.

Conc. (µg/mL)	*Mean peak area	% RSD
0	0	-
0.2	9321	0.25
0.5	23115	0.31
1	46283	0.22
2	92912	0.62
5	232159	0.84
10	461882	0.54
20	922154	0.35
40	1845001	0.41
80	3679924	0.52
100	4799591	0.49
120	5691005	0.57

*Mean of three replicates.

Table 3: Intraday precision study.

Conc. (µg/mL)	*Mean peak area
10	461882
10	461913
10	461772
10	461654
10	461811
10	461927
Mean	461826.5
SD	103.5659
% RSD	0.0224

*Mean of three replicates.

Table 4: Interday precision study.

Day	Conc. (µg/mL)	*Mean peak area
Day 1	10	461882
Day 2	10	462315
Day 3	10	463327
Mean		462508
SD		741.5814
% RSD		0.1603

*Mean of three replicates.

Table 5: Accuracy study.

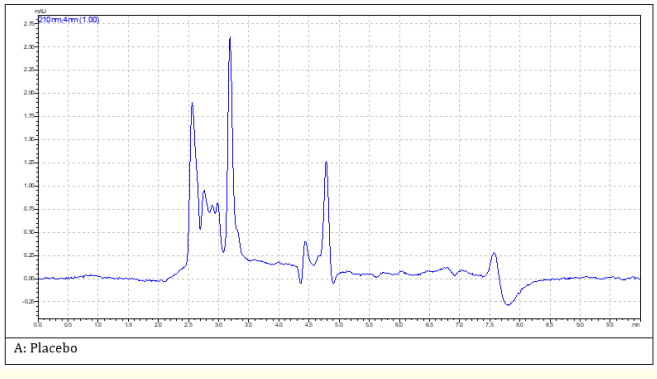
Spiked conc. (µg/mL)	Formulation (µg/mL)	Total Conc. (µg/mL)	*Conc. obtained (µg/mL) (% RSD)	% Recovery
5 5 5	10 10 10	15 15 15	14.91 (0.35)	99.40
10 10 10	10 10 10	20 20 20	19.87 (0.89)	99.35
15 15 15	10 10 10	25 25 25	24.93 (0.57)	99.72

*Mean of three replicates.

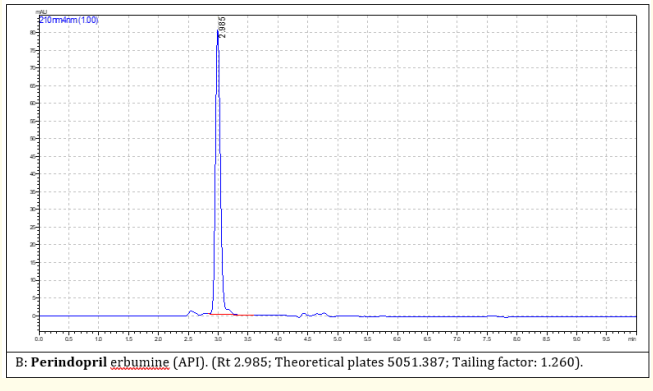
Table 6: Robustness study.

Parameter	Condition	*Mean peak area ± SD (% RSD)
Flow rate (± 0.1mL/min)	0.7 0.8 0.9	463051 ± 4306.37 (0.93)
Detection wavelength (± 2 nm)	212 210 208	461987 ± 1016.37 (0.22)
Mobile phase ratio 10 mM Tetra butyl ammonium hydrogen sulphate (pH 3.4): Acetonitrile: 0.1% Acetic acid (48: 52: 0.1) (± 2 %)	50: 50 48: 52 52: 48	462248 ± 1895.22 (0.41)
pH (± 0.05 unit)	3.45 3.40 3.35	461854 ± 4710.91 (1.02)

*Mean of three replicates.



A: Placebo



B: **Perindopril** erbumine (API). (Rt 2.985; Theoretical plates 5051.387; Tailing factor: 1.260).

Figure 2: Typical chromatograms of A) Placebo B) Perindopril erbumine (API) (10 µg/mL)

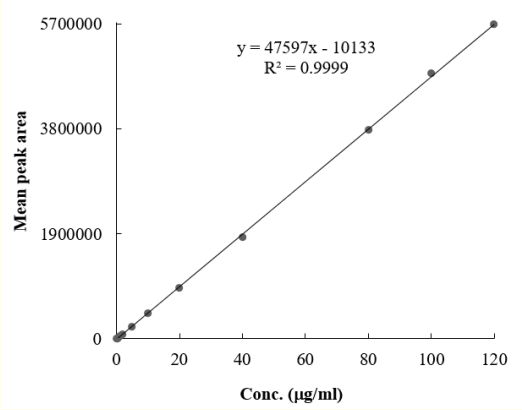


Figure 3: Calibration curve of Perindopril erbumine.

Stress degradation studies

Perindopril erbumine was eluted at 2.991 min with theoretical plates 7893 and tailing factor 0.872 During the acidic degradation Perindopril erbumine was eluted at 3.042 min with theoretical plates 7645 and tailing factor 1.307. During the alkaline degradation Perindopril erbumine was eluted at 2.926 min with theoretical plates 4268 (> 2000) and tailing factor 0.213 (Resolution 2.302) with an extra degradant peak at 3.194 min (Resolution 2.302). During oxidation reaction Perindopril erbumine was eluted at 3.035 min with theoretical plates 3726 and tailing factor 0.961 with a extra peak at 4.7 min (Resolution 9.212). During the thermal degradation Perindopril erbumine was eluted at 2.995 min with theoretical plates 4719 and tailing factor 1.161. In all the stress degradation studies the system suitability parameters were within the acceptable criteria. The details of the stress degradation studies were shown in table 7 and the corresponding chromatograms were shown in figure 4.

Table 7: Stress degradation studies.

Stress condition	R_t (min)	*Mean peak area	% Recovery	% Drug degradation	Theoretical plates	Tailing factor
Standard drug (IS)	2.991	411901	100	-	7893	0.872
Acidic hydrolysis 0.1N HCl/80ºC/30 min	3.042	396182	96.81	3.19	7645	1.307
Alkaline hydrolysis 0.1N NaOH/80ºC/30 min	2.926 3.194	354867	86.15	13.85	4268	0.21 Resolution 2.302)
Oxidation H_2O_2/80ºC/30min	3.035 4.788	368120	89.37	10.63	3726	0.961 Resolution 9.212)
Thermal degradation H_2O_2/80ºC/30 min	2.995	370535	89.96	10.04	4719	1.161

*Mean of three replicates.

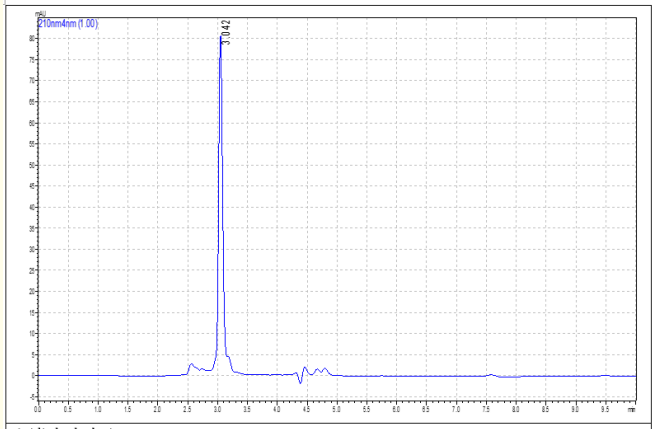
Assay of perindopril erbumine tablets

The proposed RP-UFLC method was applied to two brands of Perindopril erbumine tablets procured from different manufacturers and the assay was performed. The percentage of purity was found to be 97.75-99.25 for Perindopril erbumine (Table 8).

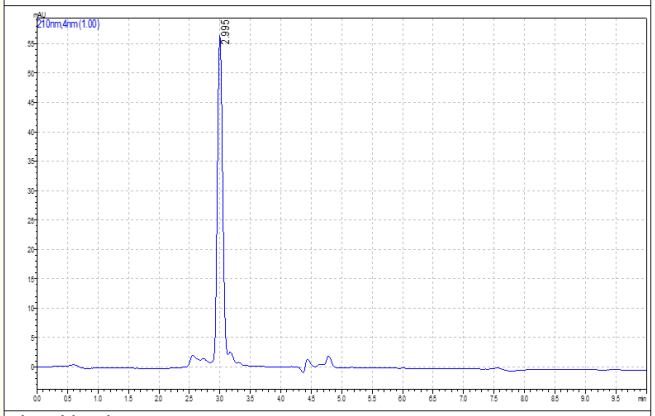
Table 8: Assay of Perindopril erbumine tablets.

Brand	Label claim (mg)	Amount found (mg)	% Assay
Brand I	4	3.91	97.75
Brand II	4	3.97	99.25

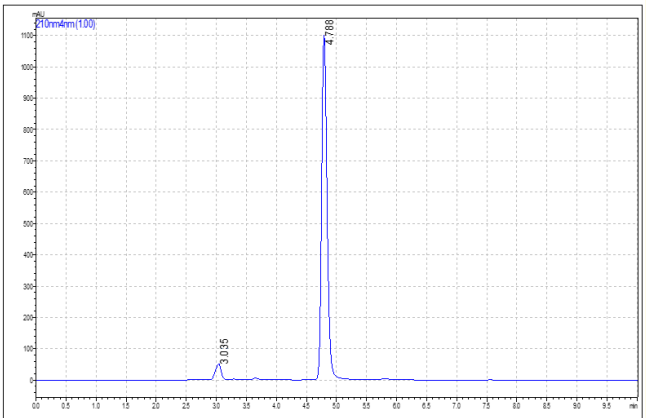
*Mean of three replicates.



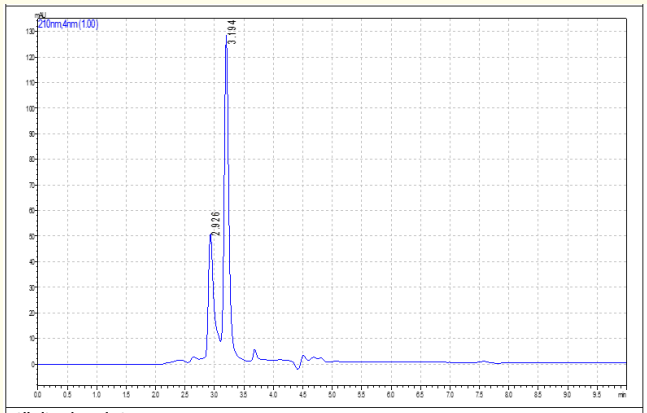
Acidic hydrolysis



Thermal degradation



Oxidative degradation



Alkaline degradation

Figure 4: Typical chromatograms of Perindopril erbumine during stress degradation studies.

Conclusion

A new stability indicating RP-UFLC method has been proposed for the estimation of Perindopril erbumine in tablet formulations. The proposed method was validated as per ICH guidelines. The method is simple, precise, accurate and robust and can be used for the regular analysis of Perindopril erbumine tablet formulations in pharmaceutical industries and no interference of excipients was observed.

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