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Determination of Losartan Potassium and Perindopril Erbumine in Tablet Formulations by Reversed-Phase HPLC

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Abstract: The present work describes a validated reverse phase high performance liquid chromatographic method for simultaneous estimation of losartan potassium and Perindopril Erbumine in tablet formulation. Chromatography was performed on a ODS Hypersil C18 (250 mm x 4.6 mm i.d., 5 µm particle size) column with mobile phase containing Acetonitrile: Acidic Water pH 3.4 (50+50). The flow rate was 1.5 mL/min and the eluent was monitored at 218 nm. The selected chromatographic conditions were found to effectively separate Losartan Potassium (RT- 4.57 min) and Perindopril Erbumine (RT- 2.31 min). Linearity for losartan potassium and Perindopril Erbumine were found in the range of 1-30 µg/mL. The values obtained of LODs were 0.182 and
0.272 µg/mL, LOQs were 0.551 and 0.826 µg/mL for losartan potassium and Perindopril Erbumine, respectively. The proposed method was found to be fast, accurate, precise, and reproducible and can be used for simultaneous analysis of these drugs in tablet formulations.
Keywords: Losartan potassium, Perindopril erbumine, Reversed-phase HPLC

INTRODUCTION

Losartan potassium (LOS) a potassium salt of 2-Butyl-4-chloro-1-[[2-(1H-tetrazol-5-yl) [1,1-biphenyl]-4-yl]methyl]-1H-imidazole-5-methanol, represents the first of a new class of orally active non-peptide angiotensin II (Type AT1) receptor antagonists employed in the management of essential hypertension [1,2] . The individual determination of losartan has been carried out in tablets by HPLC, capillary electrophoresis and super-critical fluid chromatography [3-5] in bulk and solid dosage forms by colorimetric method [6] ,in plasma by HPLC[7] , simultaneously with its degradates in stressed tablets by LC-MS/MS [8] and HPTLC [9] , with its active metabolite in biological fluids by HPLC [10-12] and in urine by gas chromatography-mass spectrometry [13].Perindopril erbumine(PER), (2S, 3(infinity) S, 7(infinity) S)-1-[(S)-N-[(S)-1-Carboxy- butyl] alanyl] hexahydro-2- indolinecarboxylic acid, 1-ethyl ester, compound with tert-butylamine (1:1), belongs to a group called angiotensin converting enzyme (ACE) inhibitors[14]. Literature surveyrevealed that few analytical methods have been reported for the estimation of PER include immunoassay [15], spectrophotometric [16-17], HPLC [18], biosensor method [19-21], LC–MS/MS [22- 23], capillary gas chromatographic method [24] The analytical method for simultaneous determination of LOS and PER in pharmaceutical formulation has not been reported so far. The present work describes a validated reverse phase HPLC method for simultaneous determination of these drugs in tablets.

Figure 1. Structure of Losartan Potassium

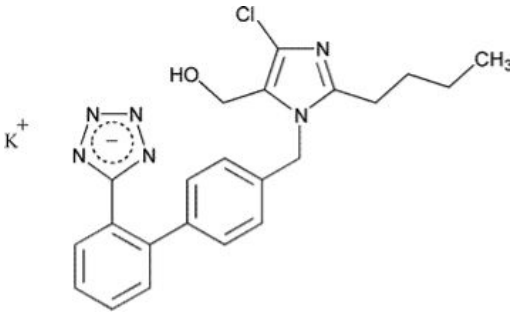
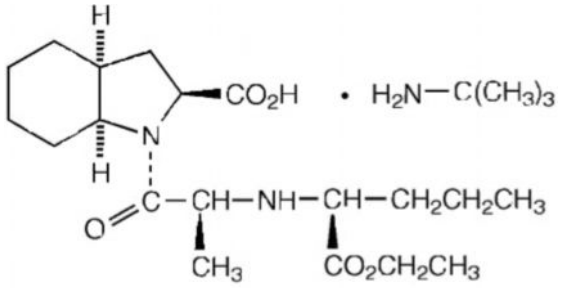


Figure 2. Structure of Perindopril Erbumine



EXPERIMENTAL

Apparatus

A Shimadzu (Columbia, MD) RP-HPLC instrument (LC-10AT vp) equipped with an photodiode array detector, manual injector with 20 µL loop, and Phenomenex (Torrance, CA) C18 column (250 mm × 4.6 mm id, 5 µm particle size) and Class-VP software were used. Sartorius CP224S analytical balance (Gottingen, Germany), and ultra sonic cleaner (Frontline FS 4, Mumbai, India) were used during the study.

Reagents and Materials

Losartan Pottasium and Perindopril Erbumide were received as a gift sample fromTorrent Research Centre, Ahmedabed, Acetonitrile of HPLC grade, Glacial acetic acid of AR grade. The water for RP-HPLC was prepared by triple glass distillation and filtered through a nylon 0.45 µm – 47 mm membrane filter (Gelman Laboratory, Mumbai,India).Pharmaceutical formulation of LOS and PER were purchased from local pharmacy.

Chromatographic Conditions

Phenomenex C18 column (250 mm x 4.6 mm i.d., 5 µm particle size) was used at ambient temperature. The mobile phase consisted of Acetonitrile: Acidic Water pH 3.4 (50+50) at a flow rate of 1.5 mL/min. The mobile phase was filtered through a nylon 0.45 µm-47 mm membrane filter and degassed before use. The elution was monitored at 218 nm, and the injection volume was 20 µL.

Preparation of solutions

Acidic water

Glacial acetic acid (2 mL) was dissolved in 1000 mL of water (pH : 3.4).

Mobile phase

Acidic water and acetonitrile in the ratio of 50:50 was used, sonicated and filtered through 0.45 µm filter.

LOS and PER Standard Stock Solutions

For HPLC analysis 5 mg of LOS and PER powder was weighed accurately using sartorius precision balance (readability 0.01 mg) and transferred in to 50 mL volumetric flask, dissolved and diluted to 50 mL with mobile phase to produce stock solution containing 100 µg/ mL of LOS and PER respectively.

Sample Solutions

20 tablets were taken and powdered. Weighed accurately a quantity of the powder equivalent to about 50 mg of LOS and 4 mg of PER into 100 mL volumetric flask and diluted to 100 mL with mobile phase. This solution is sonicated for 20 minutes. The solution was filtered through whatman filter paper No. 41. Transfer 1 mL of solution into 10 mL volumetric flask and dilute to the mark with mobile phase. Transfer 5 mL of solution into 10 mL volumetric flask and dilute to the mark with mobile phase to get a final concentration 25 µg /mL of LOS and 2µg/mL of PER.

Determination of wavelength of maximum absorbance

The standard solutions of LOS and PER were scanned in the range of 200 -400 nm against mobile phase as a blank. LOS and PER showed maximum absorbance at 218 nm. So the wavelength selected for the determination of LOS and PER was 218 nm.

Method Validation

Calibration curve (Linearity)

A calibration curves were plotted over a concentration range of 1-30 µg /mL for LOS and PER. Accurately measured standard stock solutions of LOS and PER (0.1, 0.5, 1, 1.5, 2, 2.5 and 3 mL) were transferred to a series of 10 mL volumetric flasks and the volume in each flask was adjusted to 10 mL with mobile phase. The resulting solutions were injected into the column and the peak area obtained at flow rate 1.5 mL/min for LOS and PER respectively. Calibration curves were constructed for LOS and PER by plotting peak area

versus concentration at 218 nm. Each reading was average of three determinations.

Accuracy (% Recovery)
Accuracy of the methods was assured by use of the standard addition technique, involving analysis of formulation samples to which certain amounts of authentic drugs were added. The resulting mixtures were assayed, and the results obtained for both drugs were compared to those expected. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the tablets (LOS 10 µg/mL and PER 4 µg/mL) with three different concentrations of standards (LOS 2,4,6 µg/mL and PER 2,4,6 µg/mL). The good recoveries with the standard addition method (Table 3) prove the good accuracy of the proposed methods.

Method Precision:
For evaluation of precision, repeatability of the results for a concentration of 10 µg/mL was evaluated by 6 replicate determinations. For evaluation of intermediate precision, the results over the concentration range 1 - 30 µg/mL was evaluated by 4 replicate determinations to estimate intraday variation and another replicate determination on different 4 days to estimate interday variation. The coefficients of variation (CV) values at these concentration levels were calculated.

Limit of Detection and Limit of Quantification
The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations as per International Conference on Harmonization (ICH) guidelines.
$LOD = 3.3 \times \sigma/S$
$LOQ = 10 \times \sigma/S$
Where σ = the standard deviation of the response and S = Slope of calibration curve.

Analysis LOS and PER in Combined Dosage Forms
Pharmaceutical formulation of LOS and PER was purchased from local pharmacy. The responses of formulations were measured at 218 nm for quantification of LOS and PER by using RP-HPLC. The amounts of LOS and PER present in sample solution were determined by fitting the responses into the regression equation for LOS and PER in both the methods. . Results are given in Table 4.

RESULT AND DISCUSSION
To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of Acetonitrile: Acidic Water at pH 3.2 in (50+50) ratio and 1.5 mL/min flow rate proved to be better than the other mixtures in terms of resolution and peak shape. The optimum wavelength for detection was set at 218 nm at which much better detector responses for both drugs were obtained. As it was shown in Fig. 3 the retention times were 2.31 min for PER and 4.57 min for LOS. The calibration graphs for LOS and PER were constructed by plotting the peak area versus their corresponding concentrations, good linearity for both was found over the range 1-30 µg/mL. Results obtained by applying the RP-HPLC method showed that the concentrations of LOS and PER can be simultaneously determined in prepared mixtures. The proposed method has been applied to the assay of LOS and PER in pharmaceutical dosage form. The validity of the method was further assessed by applying the standard addition technique. The results obtained indicate the additives
present do not interfere with analysis of the studied mixtures. System suitability test parameters for LOS and PER for the RP-HPLC method are reported in Table 1. The optical and regression characteristics and validation parameters are reported in Table 2.

CONCLUSION
All these factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive and rapid and can be applied successfully for the estimation of LOS and PER in bulk and in pharmaceutical formulations without interference and with good sensitivity.

Table 1: Statistical analysis of parameters required for system suitability testing of the HPLC method

System Suitability Parameters	LOS	PER
Retention Time	4.57	2.31
Tailing factor	1.18	1.26
Theoretical plate	3460	2531

Table 2: Optical and Regression characteristics and validation parameters of HPLC method for analysis of LOS and PER

Parameters	LOS	PER
Calibration range	1 - 30 µg/mL	1 - 30 µg/mL
Detection limit	0.182 µg/mL	0.272 µg/mL
Quantitation limit	0.551 µg/mL	0.826 µg/mL
Slope	73934	18207
Intercept	7449	-841
Correlation coefficient	0.9998	0.9995
Intraday RSD, %	0.31 -1.05	0.42 - 1.87
Interday RSD, %	0.36 -1.60	0.39 - 1.75

[a] Intraday and interday relative standard deviation(RSD) values of the whole concentration range

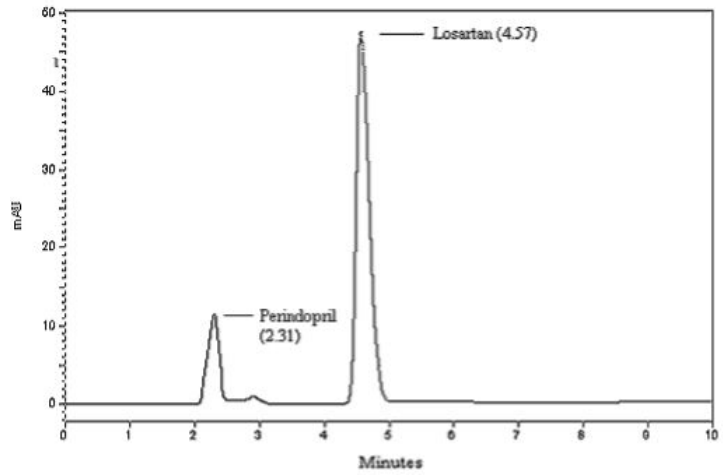
Table 3: Data of recovery study for LOS and PER by HPLC method

Drug	Amount taken (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery ± S.D (n=3)
LOS	10	2	12.21	101.75 ± 0.6438
	10	4	13.85	98.92 ± 0.4749
	10	6	15.61	97.56 ± 0.8126
PER	4	2	6.13	102.16 ± 0.7347
	4	4	8.13	101.62 ± 0.3426
	4	6	10.09	100.92 ± 0.3392

Table 4: Application of the proposed method to the pharmaceutical dosage forms

Formulation	LOS			PER		
	Amount labeled (mg)	Amount found (mg)	% Amount Found S.D. (n=3)	Amount labeled (mg)	Amount found (mg)	% Amount Found S.D. (n=3)
Brand I	50	50.46	102.92 ± 1.03	4	4.09	102.25 ± 1.16
Brand II	50	49.32	98.64 ± 1.54	4	4.03	100.75 ± 0.85
Brand III	50	49.12	99.24 ± 1.25	4	4.89	101.25 ± 1.34

Figure 3. A typical RP-HPLC chromatogram LOS (10 µg/ml) and PER (10µg/ml) with corresponding retention time



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REFERENCES

1) Smith,R.D., Chiu, A.T., Wong, P.C., Herblin, W.F., Timmermans, PBMWM, Pharmacology of Nonpeptide Angiotensin II Receptor Antagonists, Annu. Rev. of Pharmacology Toxicology,1992, 32,135-165.
2) Chiu, A.T., McCall, D.E., Price, W.A., Nonpeptide angiotensin II receptor antagonists. VII. Cellular and biochemical pharmacology of DuP 753, an orally active antihypertensive agent. J. Pharmacol. Exp. Ther..1990, 252, 711-718.
3) Williams, R.C., Alasandro, M.S., Fasone, V.L., Boucher, R.J., Edwards, J.F., Comparison of liquid chromatography, capillary electrophoresis and super-critical fluid chromatography in the determination of Losartan Potassium drug substance in Cozaar® tablets, J. Pharm. Biomed. Anal. 1996, 14, 1539-1546.
4) Maio, V.M.P., Dias, C.L., Bergold, A.M., Validation of an Isocratic HPLC Assay of Losartan Potassium in Pharmaceutical Formulations and Stress Test for Stability Evaluation of Drug Substance, Acta Farm. Bonaerense, 2005, 24, 250-255.
5) Kathiresan, K., Gothandaraman, S., Manickam, M.S., MathanKumar, S., Manavalan, R., analytical method development and validation of losartan potassium tablet by rp-hplc,,, Rasayan J. Chem. 2008, 1,521-525.
6) Prabhakar, A.H., Giridhar, R., A rapid colorimetric method for the determination of Losartan potassium in bulk and in synthetic mixture for solid dosage form, J. Pharm. Biomed. Anal. . 2002, 27(6), 861- 866.
7) Jalalizadeh, H., Souri, E.H., Farsam, M. Ansari., A High-Performance Liquid Chromatographic Assay for the Determination of Losartan in Plasma, Iranian J. of Pharmac. & Thera, 2003, 1, 18-21.
8) Zhao, Z.Z., Wang, Q., Tsai, E.W., Qin, XZand., Identification of losartan degradates in stressed tablets by LC-MS and LC-MS/MS, J. Pharm. Biomed. Anal. 1999, 20, Issues 1-2, 129-136.
9) McCarthy, K.E., Wang, Q., Tsai, E.W., Gilbert, R.E., Brooks, M.A., Determination of losartan and its degradates in COZAAR® tablets by reversed-phase high-performance thin-layer chromatography, J Pharm. Biomed. Anal, 1998, 17, 671- 677.
10) Farthing, D., Sica, D., Fakhry, I., Pedro, A., Gehr, TWB., Simple high-performance liquid chromatographic method for determination of losartan and E-3174 metabolite in human plasma, urine and dialysate, J. Chromatography B. 1997, 704 (1-2), 374-378.
11) Ritter, M.A., Furtek, C.I., Lo, M.W., An improved method for the simultaneous determination of losartan and its major metabolite, EXP3174, in human plasma and urine by high-performance liquid chromatography with fluorescence detection, J. Pharm. Biomed. Anal. . 1997,15(7), 1021-1029.
12) P.K., Jamieson, A., Smith, G.J., Fice, D., Pollak, P.T., Determination of plasma concentrations of losartan in patients by HPLC using solid phase extraction and UV detection, International journal of pharmaceutics. 2000, 204 (1-2), 17-22.
13) Maurer, H.H., Kraemer, T., Arit, J.W., Screening for the Detection of Angiotensin-Converting Enzyme Inhibitors, Their Metabolites, and AT II Receptor Antagonists, Therapeutic drug monitoring, 1998, 20 (6), 706-713.
14) Bounhoure, J.P., Bottineau, G., Lechat, P., Garnham, J., Lapeyre, G., (1989) Clin Exp Hypertens A. 11(suppl 2), 575-586.
15) Van den, B., Resplandy, G., de Bie, A.T., Floor, W., Bertrand, M., Arts, C.J., A new radioimmunoassay for the determination of the angiotensin-converting enzyme inhibitor Perindopril and its active metabolite in plasma and urine: Advantages of a lysine derivative as immunogen to improve the assay specificity, J. Pharm. Biomed. Anal. 1991, 9, 517-524.
16) Abdellatef, H. E., J Pharm Biomed Anal.1998,17, 267-270.
17) Abdellatef, H.E., Ayad, M.M., Taha, E.A., Spectrophotometric and atomic absorption spectrometric determination of ramipril and perindopril through ternary complex formation with eosin and Cu(II), J. Pharm. Biomed. Anal. 1999, 18, 1021-1027.
18) Abdalla, A., Elshanawane, Samia MM., Mohamed, S.E., Development and Validation of LC Method for Simultaneous Determination of Two Binary Mixtures Containing Indapamide, Chromatographia, 2008, 67, 837-840.
19) Stefan, R.I., van Staden, J.F., Aboul-Enein, H.Y., Determination of *S*-perindopril using a flow injection system with an amperometric biosensor, Sensors and Actuators B 1999, 54, 261- 265.
20) Van Staden, J.F., Stefan, R.I., Aboul-Enein, H.Y., Amperometric biosensor based on D-aminoacid oxidase for the R-perindopril assay, Fresenius Journal of Analytical Chemistry, 2000,367, 178-180.

21) Aboul-Enein, H.Y., Stefan, R.I., Radu, G.L., Biosensor for the enantioselective analysis of S-perindopril, Prep Biochem Biotechnol. 1999) 29, 55-61.
22) Deepak, S.J., Gunta, S., Mallika, S., Umesh, C.P., Pranav, S., First LC–MS/MS electrospray ionization validated method for the quantification of perindopril and its metabolite perindoprilat in human plasma and its application to bioequivalence study (2006) J. of Chromatography B. 837, 92-100.
23) Kandikere, R.V., Shukla, V.N., Mudigonda, M.K., Maurya, S., Komarneni. P., High-throughput quantification of perindopril in human plasma by liquid chromatography/tandem mass spectrometry: application to a bioequivalence study, Rapid Commun Mass Spectrom. (2006), 20,1864-1870.
24) Lin, S.J., Wu, H.L.,Chen, S.H., Wen, Y.H., Derivatization-gas chromatographic determination of perindopril, Analytical Letters, 1996, 29, 1751-1762.

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