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VALIDATED RP – HPLC METHOD FOR THE QUANTIFICATION OF ACE INHIBITOR PERINDOPRIL ARGININE

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ABSTRACT

A new high performance liquid chromatography (HPLC) method was developed and validated for determination of Perindopril Arginine (PA) in pharmaceutical formulations. Optimum separation was achieved in 12 minutes using C_{18} Hypersil BDS column and elution was accomplished using mobile phase with flow rate of 1 ml/min. A linear relationship between peak area and concentration of PA was observed in the range of 1 – 25 µg/ml. Precision and accuracy of the method have been established according to the current ICH guidelines. The developed method was successfully applied for the determination of PA in pharmaceutical formulations.

KEYWORDS: Perindopril Arginine; RP-HPLC; Validation; Column; ICH; Pharmaceutical Formulations.

INTRODUCTION

Perindopril Arginine, an angiotensin-converting-enzyme inhibitor (ACE inhibitor), is a pharmaceutical drug that is widely used for the treatment of high blood pressure. It can be useful for patients with stable coronary artery disease and heart failure. The main function of this ACE inhibitor is to decrease the action of the renin-angiotensin-aldosterone system in order to minimize the development of hypertension. The molecular formula of perindopril arginine and perindopril erbumine is different but their therapeutic action is very similar.[1] Perindopril is chemically known as (2*S*,3*aS*,7*aS*)-1-[(2*S*)-2-{[(2*S*)-1-ethoxy-1-oxopentan-2-yl]amino}propanoyl]-octahydro-1*H* indole-2-carboxylic acid (Figure 1).

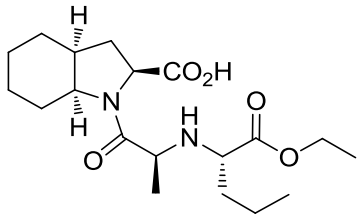

Figure 1: Structure of Perindopril.

It is a dipeptide monoacid monoester with a perhydroindole group. It has five asymmetric centers and is synthesized stereoselectively so that it is a single enantiomer (all *S* stereochemistry).[2] It is a white powder, soluble in water, slightly soluble in ethanol and practically insoluble in chloroform.[3] The perindopril arginine salt is more stable in high humidity than erbumine. It acts as a free acid and when metabolized under hydrolysis, the ester group is converted to perindoprilat, (2S,3aS,7aS)-1-[(2S)-2-[(1S)-1-carboxybutyl]amino]propanoyl]-2,3,3a,4,5,6,7,7a-octahydro-indole-2-carboxylic acid). The degradation of perindopril in pharmaceutical formulations has been studied. Diketopiperazine ethyl (2S)-2-((3S, 5aS, 9aS, 10aS)-3-methyl-1, 4-dioxodecahydro-pyrazino [1, 2-a] indol-2(1H)-yl) pentanoate was produced by cyclisation of perindopril.[4–6] According to European Pharmacopeia 6.0, this is the key degradation product known as impurity F.

The literature revealed the use of various analytical methods for the determination of perindopril, including ultraviolet spectrophotometry[7–9], high performance thin layer chromatography[10–11], high performance liquid chromatography[12–18], ultra pressure liquid chromatography[19], liquid chromatography – mass spectrophotometry[20] and fluorometry.[21] Perindopril was quantified in the presence of other main ingredients in pharmaceutical formulations; however, a buffer mobile phase and a tedious clean up procedure were needed before determination. Perindopril and its metabolites were studied and validated in human plasma using Liquid Chromatography and Mass Spectrophotometry (LC–MS).[20]

The advantage of the LC–MS method is that it produces accurate results with shorter analysis time but the method can't be used for routine work because of the high instrument's cost. The main drawback of spectroflurometric investigation of perindopril is that the analysis requires complex formation with dansyl chloride.[21] The present reported method is simple, robust with no prior clean up procedure for the determination of perindopril in pharmaceutical

formulations. The degradation was studied in the presence of acid, base and heat. The method was validated according to ICH guidelines.[22 –23] This method can be applied for routine work in the industry as well as in academic and hospitals' research labs.

1. EXPERIMENTAL

1.1. Materials

- All reagents and solvents were of HPLC grade. Methanol (Carlo Erba Reagents S.A.S. BP616, France).
- Double distilled water (Laboratory, Jubail Industrial Collge, Kingdom of Saudi Arabia).
- Coversyl and Coveram (Local pharmacy, Al - Jubail, Kingdom of Saudi Arabia).

1.2. Instrumentation

- The HPLC used was model LC – 2010 CHT, Shimadzu, Kyoto, Japan with Pump model LC - 20 AD with gegassing unit DGU – 20 A, Auto sampler model SIL - 20 AC, column oven model CTO - 20 AC.
- The detector was a PDA detector model SPD - 20 A.
- The system was driven by a HP - 5502.
- The data processing system were run with LC solution for LC – 2010 CHT (Shimadzu)
- The HPLC column used was a C_{18} 5 µm ODS hypersil column (250 mm × 4.6 mm) (Thermo Scientific, UK).

1.3. Chromatographic Conditions

The proposed method was performed using HPLC model LC - 2010 CHT (Shimadzu, Kyoto, Japan). The perindopril was eluted from the column (C_{18} 5 µm BDS hypersil, 250 mm × 4.6 mm) using methanol – water (70: 30, v/v) as a mobile phase at a flow rate of 1.0 ml/min. The mobile phase was prepared daily and degassed by passing through a 0.45 µm Ultipor filter and ultrasonication for 15 minutes. All separations were performed at 25°C with detection at 215 nm. The run time and injection volume were 12 minutes and 20 µl, respectively.

1.4. Standard Solutions

Ten tablets (claiming 5 mg of perindopril per tablet) were finely powdered. A quantity of the powder equivalent to 50 mg of PA was extracted by shaking with 100 ml of the water, followed by two extractions each with 100 ml of the mobile phase. After passing through a 0.45 µm millipore filter, the solution was diluted with mobile phase to obtain a concentration of about 50 µg/ml. It was further diluted according to the need and then analyzed following the proposed procedures.

2. METHOD VALIDATION

2.1. Specificity and selectivity

The specificity and selectivity of the proposed method were evaluated by estimating the amount of perindopril in the presence of common excipients such as anhydrous lactose, magnesium stearate, starch, lactose, glucose and fructose. The perindopril eluted from the column without any interference from the excipients.

2.2. Linearity

The linearity samples were prepared as 1, 5, 10, 15, 20 and 25 µg/ml from the standard solution. The samples were injected in the system and the results were recorded (Table 1). The linear plot was constructed between the peak area and concentration of perindopril (Figure 2). The limit of detection (LOD) and limit of quantitation (LOQ) were calculated (Table 2).

2.3. Accuracy

For the accuracy, coversyl (5 µg/ml, prepared from the standard solution) was spiked with 100%, 200%, 300% of coveram solutions in 50 ml volumetric flasks and diluted up to the mark with distilled water. The results were recorded in Table 3.

2.4. Precision

2.4.1. Intraday Precision

The perindopril (10 µg/ml) was prepared by taking 10 ml from the standard (50 µg/ml) and diluted up to 50 ml volumetric flasks with distilled water. Sample injection was done in triplicate and the % RSD was calculated (Table 4).

2.4.2. Interday Precision

The sample (10 µg/ml) was kept for seven days and then was injected in triplicate again, resulting in the peak area and % RSD shown in Table 4.

2.5. Robustness

The sample (15 µg/ml) was prepared by using 15 ml from the stock solution (50 µg/ml) into 50 ml volumetric flask and diluted with distilled water up to the mark. The sample was injected into the HPLC with the flow rate and mobile phase combination different from the normal conditions (Table 5).

2.6. Degradation studies

2.6.1. Thermal conditions

The coversyl tablets were kept for 2 hours in oven at 50°C. The tablets were then finely powered and followed by normal procedure as discussed above to make 10 µg/ml of perindopril. The sample was injected into the system and the results were presented in Table 6.

2.6.2. Hydrolytic conditions

The most common method of degradation is hydrolysis. The compound was analyzed for decomposition in the presence of acid or base (hydrochloric acid or sodium hydroxide, 0.1 – 1 M).[25 – 26] The results were recorded in Table 6.

Table 1: Linearity of perindopril from standard solution of tablet.

Concentration (µg/ml)	Peak Area
1	25981
5	31825
10	36512
15	41120
20	45232
25	51612

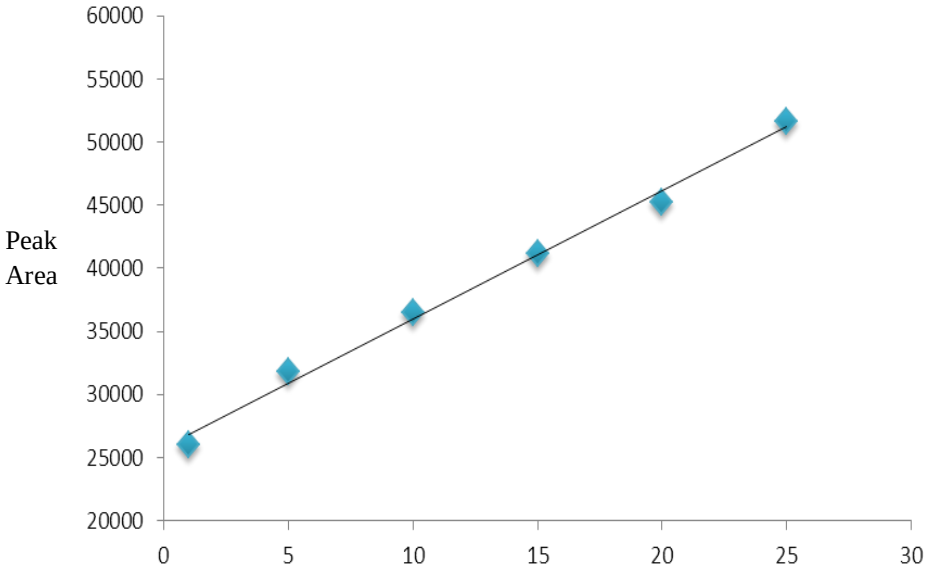

Figure 2: Linearity graph of perindopril, peak area vs concentration.

Table 2: Summary of optical and regression characteristics of the proposed method.

Parameters	Perindopril
Linearity Range (µg/ml)	1 – 25
Linear Equation	Y=1015.5X+25851
Correlation Coefficient (r^2)	0.9934
Standard Deviation (S_0)	843.76
Slope (b)	1015.47
LOD (µg/ml)	2.81
LOQ (µg/ml)	8.31

Table 3: Accuracy results of perindopril.

Concentration (ppm)	Coversyl (µg/ml)	Coveram (µg/ml)	Peak area	Found concentration (µg/ml)	RSD (%)
10	5	5	35890	10.355	0.72
10	5	5	36004	10.240	
10	5	5	35998	10.378	
15	5	10	41250	15.164	0.82
15	5	10	41021	14.938	
15	5	10	41050	14.967	
20	5	15	46102	19.942	0.88
20	5	15	46351	20.187	
20	5	15	46005	19.846	

Table 4: Intraday and interday precision of perindopril arginine sample.

Precision	Concentration (ppm)	Peak area	Found concentration (ppm)	RSD (%)
Intraday	10	36222	10.213	1.008
	10	36019	10.013	
	10	36157	10.149	
Interday	10	35988	9.982	1.084
	10	36041	10.035	
	10	36201	10.192	

Table 5: Robustness data for the perindopril tablet.

Conditions	Coveram (µg/ml)	Peak area	Found concentration (µg/ml)	RSD (%)
Change in flow rate to (0.9 ml/minute)	15	40927	14.846	0.94
	15	41102	15.018	
	15	41210	15.125	
Change in mobile phase (methanol: water = 75: 25)	15	41211	15.126	0.29
	15	41301	15.214	
	15	41255	15.169	

Table 6: Stability studies of perindopril in different conditions.

Conditions	Coveram (µg/ml)	Peak area	Found concentration (µg/ml)	RSD (%)
Thermal	20	45201	19.055	0.15
	20	45250	19.102	
	20	45199	19.053	
Acid	20	46587	20.420	1.08
	20	46610	20.442	
	20	46987	20.813	
Base	20	46002	19.843	1.41
	20	46045	19.885	
	20	46520	20.354	

3. DISCUSSIONS

Stability and degradation studies of quality control samples are very important for the development and determination of pharmaceutical formulation product. The current method was validated according to ICH guidelines and it required the combination of methanol, water as a mobile phase and 12 minutes for the analysis. The method is very useful for the determination of perindopril in a quality control laboratory as well as in standard research laboratories.

The linearity was over the concentration range of 1 - 25 µg/ml. The linear equation Y = 1015.5x + 25851, was obtained, where Y is peak area and X is concentration in ppm (µg/ml). The accuracy of the method was found in the range of 0.72 – 0.88%. The precision results are in the limit of ICH and were found to be in the range of 1.008 – 1.084%. The robustness of the method was also confirmed. Changes in mobile phase and flow rate had no effect on the analysis; the % RSD were in the range of 0.29 – 0.94%. The formulation products are stable after adding acid or base. There were no interferences from the acid and base and the solution was stable for analysis over the period of one month.

4. CONCLUSION

A simple, rapid, accurate and precise HPLC method was developed for the determination of perindopril arginine in pharmaceutical tablets. The analytical conditions developed provided a good separation for perindopril arginine within a short analysis time. The validated method demonstrated a wide linear dynamic range with good precision and accuracy. Thus, the method can be proposed for routine analysis as well as for quality control laboratories.

REFERENCES

1. Economics legislation committee, Commonwealth of Australia, Creative Commons Attribution-Noncommercial-No Derivs, Parliament House, Canberra, Australia, 2015.
2. Vincent, M; Remond, G; Portevin, B; Serkiz, B; Laubie, M. Stereoselective synthesis of a new perhydroindole derivative of chiral iminodiacid, a potent inhibitor of angiotensin converting enzyme. *Tetrahedron Lett*., 1982; *B23:* 1677 – 1680.
3. Rui. X.; Liang-Yin, C.; Jin-Gen, D. Membranes and membrane processes for chiral resolution. Chem. Soc. Rev., 2008; *37:* 1243 – 1263.
4. Grislain, L; Mocquard, M.T; Dabe, J.F; Bertrand, M; Luijten, W; Marchand, B; Resplandy, G;

Devissaguet, M; Interspecies comparison of the metabolic pathways of perindopril, a new angiotensin-converting enzyme (ACE) inhibitor. *Xenobiotica*, 1990; *20*: 787 – 800.
5. European Pharmacopoeia 6.0, vol. 2, January 2008 (monograph perindopril tert-butylamine).
6. Medenica, M; Ivanovic, D; Maskovic, M; Jancic, B; Malenovic, A. Evaluation of impurities level of perindopril *tert*-butylamine in tablets. *J. Pharm. Biomed. Anal*., 2007; *44:* 1087–1094.
7. Ratnaparkhi, M.; Patil, P.; Jagadale, S. Development and validation of spectrophotometric method for the determination of perindopril erbumine in bulk dosage form. *American J. Phytomed. Clin. Therap*., 2013; *1*: 78 – 82.
8. Avula, P. R.; Chatragadda, N.; Sunkara, B.; Chandra, B. S. Determination of perindopril erbumine in tablets by spectrophotometry. *Jordan J. Chem.*, 2012; *7:* 413 – 426.
9. Jignesh, P. P.; Patel, M. B., Rashmika J. P.; Nimesh, A. P. Simultaneous determination of perindopril erbumine and amlodipine besylate by absorption factor method. *Int. J. Appl. Biol. Pharma. Tech.*, 2011; *2*: 230 – 233.
10. Varsha, B; Viraj, H; Mrinalini, C.D. Development and validation of stability indicating HPTLC method for the estimation of perindopril and indapamide. Int. J. Pharm. Pharma. Sci., 2014; 6: 621 – 625.
11. Dewani, M.G; Bothara, K.G., Damle, M.C. Development and validation of stability indicating HPTLC method for the determination of perindopril erbumine. *Int. Res. J. Pharm.*, 2010; *1:* 428 – 435.
12. Saravanan, G; Kommara, D. A. J; Krishnanjaneyulu I.S; Visagaperumal, D. Stability indicating RP-HPLC method for simultaneous estimation of Amlodipne and Perindopril in bulk and formulation. *J. Chem. Pharma. Res.*, 2014; *6:* 465 – 473.
13. Rao, M.V; Rao, D. N; Rao, M. P; Eswarudu, M.M. RP – HPLC method development and validation for simultaneous estimation of losartan potassium and perindopril erbumine in pharmaceutical dosage form. *Int. Res J Pharm. App Sci*., 2013; *3:* 286 – 292.
14. Gumieniczek, A; Mączka, P; Inglot, T; Pietras, R; Lewkut, E; Perczak, K. New HPLC method for in vitro dissolution study of antihypertensive mixture amlodipine and perindopril using an experimental design. *Cent. Eur. J. Chem.*, 2013; *11:* 717 – 724.
15. Gumustas, M; Ozkan, S.A. A validated stability-indicating RP-LC method for the simultaneous determination of amlodipine and perindopril in tablet dosage form and their stress degradation behavior under ICH-recommended stress conditions. *J. AOAC Int.*, 2013; *96*: 751 – 757.
16. Zaazaa H.E; Abbas, S.S; Essam, H.A.M; El-Bardicy, M.G. Validated chromatographic methods for determination of perindopril and amlodipine in pharmaceutical formulation in the presence of their degradation products. *J. Chromatogr. Sci.*, 2013; *51*: 533 – 543.
17. Raju, V.B; Rao, A.L. Development and validation of new HPLC method for the estimation of perindopril in tablet dosage forms. *Rasayan J. Chem*., 2011; *4:* 113 – 116.
18. Rani, P. A; Sekaran, B. C. A validated RP - HPLC method for the determination of perindopril erbumine in pharmaceutical formulations. *Int. J. Pharm Tech Res.*, 2009; *1*: 575 – 578.
19. Paczkowska, M; Zalewski, P; Garbacki, P; Talaczynska, A; Krause, A; Cielecka‐Piontek, J. The development and validation of a stability‐indicating UHPLC‐DAD method for determination of perindopril L‐arginine in bulk substance and pharmaceutical dosage form. *Chromatographia*, 2014; *77:* 1497 – 1501.
20. Jain, D.S; Subbaiah, G; Sanyal, M; Pande, U.C; Shrivastav, P. 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. *J. Chromatogr. B.*, 2006; *837*: 92 – 100.
21. Sakur, A.A; Chalati, T; Fael, H; Selective spectrofluorimetric method for the determination of perindopril erbumine in bulk and tablets through derivatization with dansyl Chloride. *J. Anal. Sci. Tech.*, 2015; *6*: 12, DOI 10.1186/s40543-015-0045-6.
22. International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use, validation of analytical procedures: text and methodology Q2 (R1), Geneva, Canada, 27 October 1994.
23. International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use, stability testing: photostability testing of new drug substances and products Q1B, Geneva, Canada, 6 November 2006.