Research Article

Hacettepe University Journal of the Faculty of Pharmacy
Volume 44 / Number 4 / December 2024 / pp. 306-317

Developing and Validation of a High-Performance Liquid Chromatography Method for the Determination of Combined Perindopril, Indapamide and Amlodipine from Pharmaceutical Preparations

Sevinç Ayla ÖZSAR[1,2]
ORCID: 0009-0004-2759-4790
Sacide ALTINÖZ[3]*
ORCID: 0000-0003-2864-9218

[1]Ankara University, The Graduate School of Health Sciences, 06110, Ankara, Türkiye

[2]Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, 06560, Ankara, Türkiye

[3]Başkent University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, Türkiye

Corresponding author:
Sacide ALTINÖZ
Baskent University, Faculty of Pharmacy, Department of Analytical Chemistry
Ankara, Turkey
E-mail: sacidealtinoz@baskent.edu.tr
Tel: +90 312 246 66 66

Received date : 01.03.2024
Accepted date : 20.10.2024

DOI: 10.52794/hujpharm.1445884

ABSTRACT

High Performance Liquid Chromatographic method was developed and validated for the analysis of a pharmaceutical preparation (Triplixam®) consist of Perindopril (PRN), Indapamide (IND) and Amlodipine (AML) which are used for the control of hypertension in patients with high blood pressure. In this developed HPLC method, ACE 5 C18 analytical column (12.5 x 4.6 mm) was used for the analysis of PRN, IND and AML. Acetonitrile and 50 mM phosphate buffer mixture (40:60 v/v) was used as mobile phase. Measurements were obtained at 215 nm wavelength using UV detector. Retention times of PRN, AML and IND were 2.22, 3.48 and 5.05 min. respectively, at selected chromatographical conditions. The developed HPLC method was validated in terms of stability, accuracy, sensitivity, linearity, precision, specificity, ruggedness and robustness in accordance with ICH analytical method. Linear calibration curves were obtained in the range of 1.0-25.0 ppm; 1.0-8.0 ppm and 1.0-40.0 ppm for PRN, IND and AML, respectively. In this HPLC method the limit of detection values (LOD) for PRN, IND and AML were 0.25 ppm, 0.10 ppm and 0.15 ppm while limit of quantification values (LOQ) were 0.50 ppm, 0.25 ppm and 0.40 ppm, respectively.

Keywords: Amlodipine, High Performance Liquid Chromatography, Indapamide, Perindopril, Validation

1. Introduction

Hypertension is a public health problem because it causes serious complications and has a high incidence in the community. In the treatment of essential hypertension, the active ingredients of Perindopril (PRN), Indapamide (IND) and Amlodipine (AML) are used simultaneously or in combinations. Each of the active ingredients lowers blood pressure and works together to control blood pressure.

PRN inhibits an angiotensin converting enzyme (ACE) and increases bradykinin levels. Thus, vasoconstriction is prevented, vasodilation occurs and peripheral vascular resistance decreases.

By inhibiting the reabsorption of sodium in the cortical dilution segment, IND increases urinary output indirectly by increasing the excretion of sodium, chlorine and, to a lesser extent, potassium and magnesium, thus an antihypertensive effect is observed. AML, on the other hand, decreases the systolic calcium level by reducing the entry of calcium ions into the myocardial cells to the cardiac and vascular smooth muscles, and exerts an antihypertensive effect by reducing peripheral resistance (Figure 1).

Pharmaceutical preparation containing a combination of these three antihypertensive active ingredients with complementary mechanisms, used to control blood pressure in patients with hypertension [1,2].

There are studies on the analysis of Perindopril, Indapamide and Amlodipine with different methods than pharmaceutical preparations alone. Among these methods, there are studies on Perindopril using the liquid chromatography method [3-6] and similarly, there are studies on the analysis of Indapamide from pharmaceutical preparations by liquid chromatography alone [7-8]. However, there were no studies on the analysis of Amlodipine from pharmaceutical preparations by liquid chromatography alone.

However, studies on the analysis of various combinations of amlodipine, one of the pharmaceutical preparations, with different active substances by liquid chromatography are frequently encountered [9-19]

On the other hand, there are studies on the analysis of the mixture of Perindopril with different active substances from pharmaceutical preparations by liquid chromatography [20].

Moreover; There are studies on the analysis of combinations of indapamide with different active substances by liquid chromatography [21-27].

There are also many studies on the dual combinations of the active ingredients of Perindopril, Indapamide, Amlodipine. Studies on the analysis of Perindopril-Amlodipine mixture by liquid chromatography [28-32], studies on the analysis of Perindopril-Indapamide mixture by liquid chromatography [33-38] and studies on the analysis of Amlodipine-Indapamide mixture by liquid chromatography [39-41] are frequently encountered in the sources.

The simultaneous determination of Perindopril, Indapamide and Amlodipine allows the analysis of the triple combination tablet with only one analytical method, thus saving time, effort and mobile phase consumption compared to analyzing each substance separately. In this context, the main purpose of this study is simultaneous analysis of the triple drug mixture of Perindopril, Indapamide and Amlodipine from pharmaceutical preparations with the high performance liquid chromatography (HPLC) method. Analysis conditions will be optimized for the determination of Perindopril, Indapamide and Amlodipine mixture by HPLC, and a method that allows

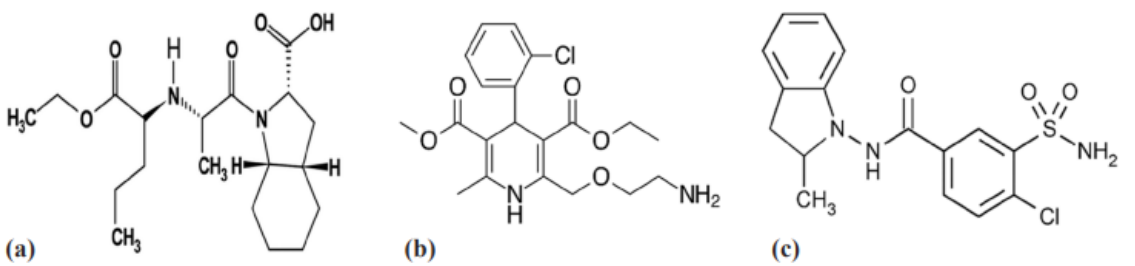

Figure 1. Structure of (a) PRN, (b) AML, (c) IND

ISSN: 2458 - 8806

the analysis of this mixture from a pharmaceutical preparation (tablet) will be developed.

In the validation of the developed method, the method validation parameters required in the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guideline [42] and The United States Pharmacopeia (USP) [43] were tested and the results evaluated statistically.

There are liquid chromatography methods developed for the simultaneous analysis of Perindopril, Indapamide and Amlodipine in the literature [44-49], however, in this study, a new methodology was created and validated, aiming to increase the analytical performance and improve the chromatographic separation of Perindopril, Indapamide and Amlodipine, compared to other liquid chromatography methods. Further discussion on the advantages and disadvantages of other studies compared to our study will be included in the following sections.

2. Material and Methods

2.1. Chemicals

Acetonitrile (ACN, HPLC grade) and methanol were purchased from Iso-Lab, while potassium dihydrogen phosphate (KH_2PO_4, analytical grade**)** from Merck. Ultrapure water was obtained from Barnstead NanoPure Diamond System. PRN, IND, AML active pharmaceutical ingredients and NPX which is used as internal standard (IS), were purchased from Merck. Triplixam® 5 mg/1.25 mg/10 mg tablets were purchased from local pharmacies.

2.2. Instrumentation and chromatographic conditions

Thermo Separation Products System HPLC system consisting diode array detector was used for chromatographic analysis. 215 nm wavelength was chosen as detector wavelength. Analysis was performed using ACE C18 (125 × 4.6 mm, 5 µm) analytical column. Flow rate of 1.0 mL/min and injection volume of 5 µL were set. Column oven temperature was set to 35 °C. NaH_2PO_4 (pH 4.6; 50 mM), and EtOH (95:5, v/v) utilized as mobile phase. System suitability parameters as capacity factor (k'), selectivity (α), plate number (N), and resolution (Rs), and tailing factor parameters (T) were inspected according to acceptance criteria of k'>2, RSD≤ 1, N > 2000, Rs > 1.5, T<2.

2.3. Preparation of stock solutions and working solutions

PRN, IND and AML stock solutions were prepared in ACN while IS prepared in methanol. Working solutions were prepared daily in desired concentrations in the mobile phase with addition of 10 µL of the internal standard (IS).

2.4. Preparation of synthetic tablet solutions and tablet samples

Calcium Carbonate, Pregelatinized Starch, Microcrystalline Cellulose (E 460), Croscarmellose Sodium (E 468), Magnesium Stearate (E 572), Colloidal Anhydrous Silica, Glycerol (E 422), Hypromellose 6mPa.s (E 464), Macrogol 6000 and Titanium Dioxide (E 171) was weighed in certain amounts and a synthetic tablet solution was prepared by adding known amounts of standard PRN, IND and AML (5 mg PRN / 1.25 mg IND / 10 mg AML) to this mixture.

10 tablets are weighed and ground into powder in a mortar. One tablet weight of this obtained tablet powder is weighed and dissolved with ACN in a 50 mL flask. Thus, a solution containing 100 ppm PRN, 25 ppm IND, and 200 ppm AML is obtained. From this filtered solution, 80 µL is taken into each vale and after adding 20 µl of the internal standard stock solution, the total volume is made up to 1000 µL with the mobile phase.

3. Results and Discussion

In the simultaneous analysis of PRN, IND and AML from the pharmaceutical preparation with HPLC; retention time, capacity factor, separation, number of theoretical layers and peak symmetry ratio are the chromatographic parameters that are considered in determining the appropriate chromatographic conditions.

Optimum conditions were tried to be determined by examining how the changes in column and filler, mobile phase organic solvent ratio, mobile phase pH, mobile phase flow rate and injection volume affected these chromatographic parameters. In the validation studies of the developed method, validation param-

eters such as stability, specificity, linearity, accuracy and precision, sensitivity (lower and upper detection limits), recovery, robustness and ruggeddness parameters were evaluated on the basis of ICH guideline regulations [42] and USP requirements for pharmaceutical analysis [43].

3.1. System suitability

System suitability study was performed with 6 repetitive injections of PRN, IND and AML standard solutions and the results of the peaks are given in Table 8. The Relative Standard Error (RSD) values for injection precision of 6 replicate experiments were found as 0.63, 0.64 and 0.59 respectively expressing high precision. Peak asymmetry factor of 0.99, 1.03 and 0.99, theoretical plate number of 2159, 3254 and 3481 were found respectively. Resolution between PRN and AML was 2.38 and 1.99 between AML and IND which should be at least 1.5. All the parameters for PRN, IND and AML from tablet was compatible with USP [43].

3.2. Selectivity

Chromatograms of prepared placebo, standard, synthetic and pharmaceutical preparation solutions were examined. The fact that the retention times of the peaks of the substances and the internal standard are the same and no other peaks are observed, shows that the matrix does not affect the analysis and the method is selective (Figure 2).

Also, in search for matrix effect on the quantitative analysis of the active pharmaceutical ingredients, standard addition method is used. Since the slopes of the lines obtained from the standard addition method and the slopes of the lines obtained from the calibration method are very close to each other, calibration lines were used in the analysis of pharmaceutical preparations.

3.3. Linearity

The ratio of PRN, IND and AML peak areas to the internal standard (NPX) area against PRN, IND and AML concentrations was plotted and linear calibration curves were obtained (Table 1).

3.4. Accuracy and precision

The methodology used to conduct accuracy and precision studies based on intra-day and inter-day measurements. The study involved analyzing solutions of PRN, IND, and AML at different concentrations. Intra and inter-day accuracy and precision were assessed by preparing and analyzing six different solutions at three concentrations on the same day and on six consecutive days (Table 2). Accuracy was determined by calculating the % relative error, which compared the amount of substances added to the amount found. Precision was assessed using mean ± standard error, standard deviation, and percent relative error values. Additionally, precision was demonstrated through injection and method repeatability tests, as shown in Table 3, where the methods exhibited a strong level of precision, evident from standard deviations measuring under 2.

3.5. Sensitivity

Limit of Detection (LOD) was taken as the lowest detectable concentrations of PRN, IND and AML when the signal-to-noise (S/G) ratio was 3. Limit of Quantification (LOQ) was taken as the lowest detectable PRN, IND and AML concentrations when the (S/G) ratio was 10 (96) (Table 1).

The LOD values of PRN, IND and AML analyzed by HPLC were 0.25 ppm, 0.10 ppm and 0.15 ppm and the LOQ values were 0.50 ppm, 0.25 ppm and 0.40 ppm, respectively.

3.6. Ruggedness and robustness

In order to demonstrate the robustness of the developed HPLC method, when small changes were made in the method parameters, it was examined to what extent the findings were affected by these changes. The results obtained as a result of changes in ACN ratio, mobile phase pH, buffer concentration and flow rate were compared with the results obtained in the optimum condition and no difference was observed. In the light of this information, the developed HPLC method was proved to be robust to small changes in the method parameters, i.e. no change in any way (Table 4).

To determine whether the method was consistent, the difference between the results of the analyses performed by two different analyzers was statistically compared. As a result, it was found that the difference between the 1st and 2nd analyzer was not statistically significant, proving the consistency of the developed HPLC method Table 5, $p > 0.05$.

ISSN: 2458 - 8806

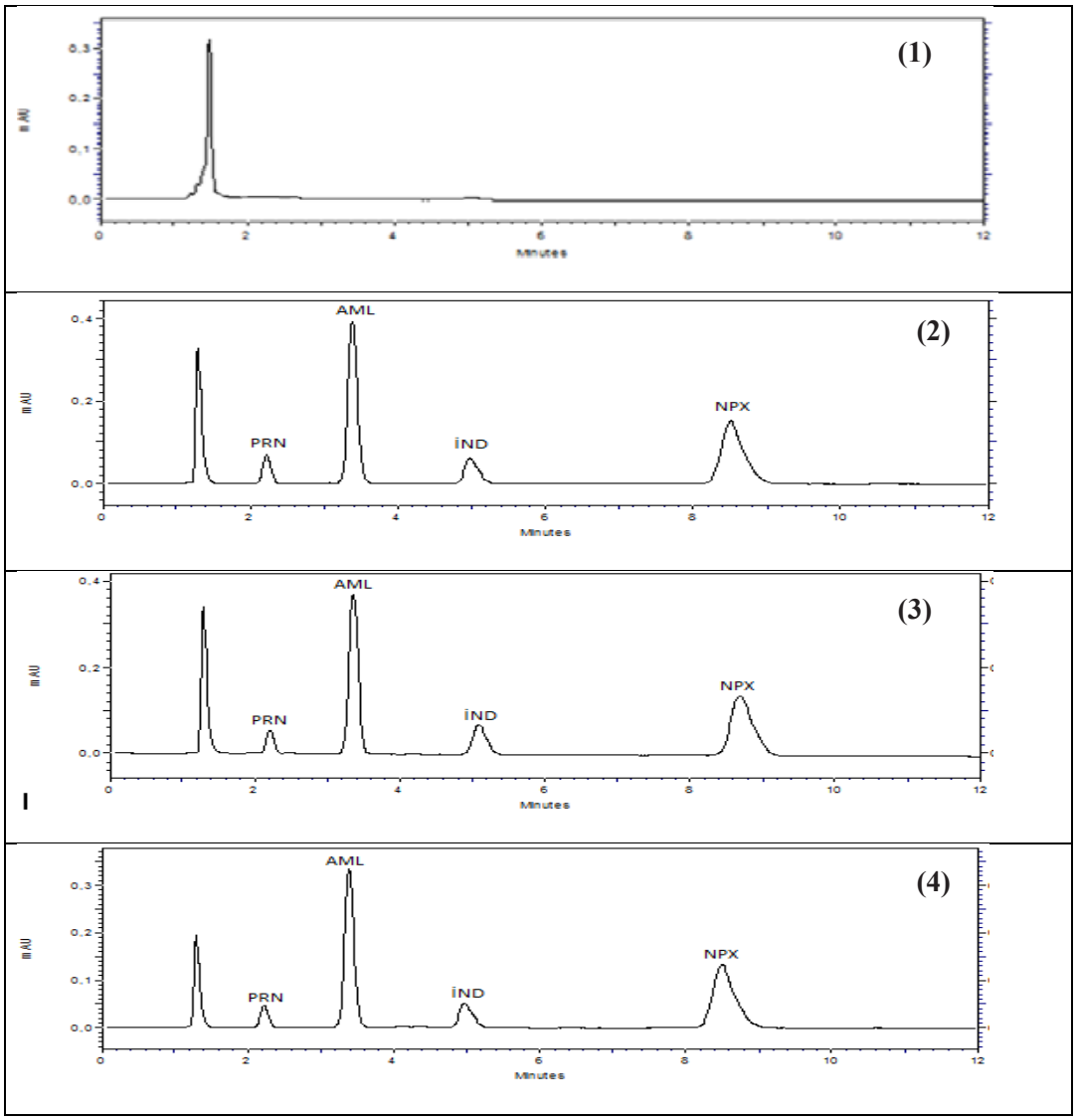

Figure 2. Chromatograms of (1) placebo), (2) standards ve IS (3), Synthetic Tablet, (4) Pharmaceutical preparation (tablet); PRN (8 ppm), AML (16 ppm) ve IND (2 ppm). Chromatographic Conditions: ACN: 50 mM phosphate buffer (40:60, v/v), pH: 3.0, Flow rate: 1.0 mL/min., Injection volume: 20 µL.

Table 1. Characteristics of calibration curves

	PRN	**AML**	**IND**
Regression Equation	y=0.0206x+0.0139	y=0.0516x+0.0356	y=0.1141x+0.0231
Correlation Coefficient (R^2)	0.9987	0.9992	0.9999
Linearity Range (ppm)	1.0 – 25.0	1.0 – 40.0	1.0 – 8.0
LOD, Limit of Dedection (ppm)	0.25	0.15	0.10
LOQ, Limit of Qantification (ppm)	0.50	0.40	0.25

Table 2. Accuracy and precision values of the developed methods for PRN, IND and AML from tablet

Added PRN (ppm, n=6)	Intra-day	Inter-day
	Found (ppm)	Found (ppm)
2 ppm		
$\bar{X}$	1.98 ± 0.01	1.99 ± 0.01
SD	0.01	0.01
RSD (%)	0.65	0.71
5 ppm		
$\bar{X}$	5.06 ± 0.00	4.98 ± 0.03
SD	0.01	0.06
RSD (%)	0.21	1.31
15 ppm		
$\bar{X}$	15.23 ± 0.02	15.15 ± 0.06
SD	0.06	0.15
RSD (%)	0.36	0.10

Added IND (ppm, n=6)	Intra-day	Inter-day
	Found (ppm)	Found (ppm)
2 ppm		
$\bar{X}$	2.03 ± 0.00	2.02 ± 0.00
SD	0.00	0.01
RSD (%)	0.20	0.50
4 ppm		
$\bar{X}$	4.05 ± 0.01	4.05 ± 0.01
SD	0.03	0.02
RSD (%)	0.67	0.63
6 ppm		
$\bar{X}$	6.02 ± 0.00	6.05± 0.02
SD	0.01	0.05
RSD (%)	0.16	0.76

ISSN: 2458 - 8806

Added AML (ppm, n=6)	Intra-day	Inter-day
	Found (ppm)	Found (ppm)
2 ppm		
$\bar{X}$	1.99 ± 0.00	2.00 ± 0.01
SD	0.01	0.01
RSD (%)	0.53	0.70
8 ppm		
$\bar{X}$	8.02 ± 0.02	8.01± 0.04
SD	0.04	0.09
RSD (%)	0.53	1.17
25 ppm		
$\bar{X}$	25.11 ± 0.01	25.14 ± 0.15
SD	0.01	0.36
RSD (%)	0.54	1.44

$\bar{X}$: Mean ±Standard error, SD: Standard Deviation, RE: Relative error, RSD: Relative Standard Deviation

Table 3. Method repeatability findings of the developed HPLC method (analyzed PRN: 5 ppm, IND: 4 ppm, AML: 8 ppm, NPX: 4 ppm, n=10)

PRN	IND	AML
Found (ppm)	Found (ppm)	Found (ppm)
4.97±0.01 SD 0.03 RSD %0.65	4.00±0.02 SD 0.05 BSS %1.34	8.02±0.03 SD 0.09 BSS %1.08

$\bar{X}$: Mean ± Standard error, SD: Standard Deviation, RSD: Relative Standard Deviation

Table 4. Robustness results of tablet analysis (Added 5 ppm PRN, 4 ppm IND ve 8 ppm AML, 4 ppm NPX, n=3)

Parameters	Found	%BSS	p value
Optimum conditions	9.94 ± 0.06	0.99	-
Flow rate 0.9 mL/min	9.99 ± 0.10	1.66	0.67
Flow rate 1.1 mL/min	10.00 ± 0.06	1.05	0.51
Column temperature 33 °C	9.98 ± 0.04	0.67	0.57
Column temperature 37 C	9.86 ± 0.03	0.45	0.27
Buffer concentration 47.5 mM	9.95 ± 0.08	1.39	0.95
Buffer concentration 52.5 mM	10.06 ± 0.04	0.76	0.19
Acetonitrile ratio 36 %	9.88 ± 0.06	0.99	0.47
Acetonitrile ratio 44 %	9.94 ± 0.07	1.19	0.98

The results were compared with the results obtained under optimum conditions ($p > 0.05$).

Table 5. Ruggedness results of tablet analysis (Added 5 ppm PRN, 4 ppm IND ve 8 ppm AML, 4 ppm NPX)

	Injection Repeatability for PRN	
	Analyst 1	**Analyst 2**
Added (μg mL^{-1})	5	5
$\bar{X}$	5.01±0.03	5.01±0.01
SD	0.07	0.02
RSD	1.32	0.36
Wilcoxon Test	$T_H = 11 > T_T = 2$	
	Injection Repeatability for IND	
	Analyst 1	**Analyst 2**
Added (μg mL^{-1})	4	4
$\bar{X}$	3.96±0.02	3.98±0.02
SD	0.05	0.05
RSD	1.17	1.28
Wilcoxon Test	$T_H = 15 > T_T = 2$	
	Injection Repeatability for AML	
	Analyst 1	**Analyst 2**
Added (μg mL^{-1})	8	8
$\bar{X}$	7.98±0.04	7.98±0.05
SD	0.11	0.12
RSD	1.38	1.47
Wilcoxon Test	$T_H = 12 > T_T = 2$	

$\bar{X}$: Mean ± Standard error, SD: Standard Deviation, RE: Relative error, RSD: Relative Standard Deviation

3.7. Recovery

Recovery values of synthetic preparation solution containing 5 mg PRN, 1.25 mg IND, 10 mg AML and excipients are given in Table 6.

3.8. Application to tablet analysis

The results obtained from the analysis of PRN, IND and AML from the pharmaceutical preparation by HPLC are presented in Table 7.

3.9. System suitability test

The system suitability test was performed with 6 replicate injections of PRN, IND and AML standard solutions. System suitability parameters for PRN, AML and IND peaks are given in Table 8.

4. Conclusion

HPLC method has been developed for the determination of PER, IND and AML simultaneously in pharmaceutical dosage form. The developed HPLC method was validated in terms of stability, accuracy, sensitivity, linearity, precision, specificity, ruggedness and robustness in accordance with ICH Q2 (R2) guideline.

Within the scope of the validation studies, validation parameters such as stability of analyte solutions, specificity, linearity, accuracy and precision, lower and upper limits of determination, recovery, reproducibility, robustness and ruggedness were evaluated and it was found that all parameters met the acceptance criteria. Linear calibration curves were obtained

ISSN: 2458 - 8806

Table 6. Results of PRN, IND and AML amount from synthetic tablet (Added 5 mg PRN, 1.25 mg IND ve 10 mg AML, n=6)

	PRN (5 mg)	IND (1.25 mg)	AML (10 mg)
$\bar{X}$	5.00±0.01	1.26±0.004	10.03±0.03
SD	0.03	0.01	0.06
RSD	0.65	0.74	0.66

$\bar{X}$: Mean ± Standard error, SD: Standard Deviation, RE: Relative error, RSD: Relative Standard Deviation

Table 7. Results of analysis of pharmaceutical preparations (n=6)

Triplixam 5 mg/1.25 mg/10 mg Film Coated Tablet			
	PRN (5 mg)	**IND (1.25 mg)**	**AML (10 mg)**
$\bar{X}$	5.00±0.01	1.25±0.003	9.98±0.02
SD	0.02	0.01	0.05
RSD	0.39	0.62	0.53

$\bar{X}$: Mean ±Standard error, SD: Standard Deviation, RE: Relative error, RSD: Relative Standard Deviation

Tablo 8. System suitability parameters for PRN, AML and IND peaks

	PRN	PRN-AML	AML	AML-IND	IND
Retention Time (dk)	2.22		3.48		5.05
Retentipn Time % BSS	0.10		0.20		0.10
Enjection Repeatability	0.63		0.64		0.59
Capacity Factor	0.76		1.78		3.04
Resolution		2.38		1.99	
Symmetry Factor	0.99		1.03		0.99
Number of Theoretical Plates	2159		3254		3481

in the range of 1.0-25.0 ppm for PRN, 1.0-8.0 ppm for IND and 1.0-40.0 ppm for AML. The correlation coefficients close to 1.0000 indicate that the calibration curves obtained are linear. The LOD and LOQ values indicate that the developed HPLC method is highly sensitive for the analysis of the pharmaceutical preparation. In intra-day and inter-day accuracy and precision studies, % RE (relative error) values less than 2% prove the accuracy of the method and % RSD (relative standard deviation) values less than 1.5% prove the precision of the method. In addition, the ruggedness and robustness of the method has been demonstrated.

Compared to other studies (44-47), the LOD and LOQ values obtained in our study (The LOD values of PRN, IND and AML were 0.25, 0.10 and 0.15 ppm; the LOQ values were 0.50, 0.25 and 0.40 ppm, respectively) showed that the sensitivity

of the method we developed was much higher. At the same time, the method we developed provides advantages in terms of both time and cost due to its shorter analysis time compared to the study by Chaudhary et al. [45] and the study by Patel et al. [48]. On the other hand, in the study conducted by Karadurmus et al. [49], the capacity factor values were determined as 0.5, 1.02 and 1.92 for PRN, AML and IND, respectively. In our study, the capacity factor values were determined as 0.76, 1.78 and 3.04 for PRN, AML and IND, respectively. As is known, in the liquid chromatography method, a high capacity factor means that elution takes a very long time, while a low capacity factor means that there is a low level of interaction between the substance and the stationary phase. In this context, when compared with the study conducted by Karadurmus et al. [49], the method we developed showed a good improvement in the capacity factor. In addition, the internal standard method was used in our study, and thus any loss in the sample during analysis was balanced with an equal loss of the internal standard, thus reducing systematic or unsystematic errors in analytical measurements. None of the studies [44-49] on the simultaneous analysis of the triple combination of PRN, AML, IND by liquid chromatography used internal standards. In this respect, the method we developed provides advantages over other methods.

With the developed method, PRN, IND and AML were successfully analyzed simultaneously from combined dosage form. The proposed method can be applied for analysis of combined dosage form, allowing the savings of time, effort, and cost. Based on its frequent use in R&D and routine analysis laboratories, the developed HPLC method is recommended to be used for routine analysis in laboratories in the pharmaceutical industry and all other related fields.

Conflict of Interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

Statement of Contribution of Researchers

SA and SAO are conceived and designed the analysis. SAO performed the analysis. SA and SAO wrote the paper and approved the manuscript.

References

1. Servier İlaç ve Araştırma A.Ş. [cited June 2016]. Avaliable from: http://www.servier.com.
2. Türk Kardiyoloji Derneği. Türk Kardiyoloji Derneği Ulusal Hipertansiyon Tedavi ve Takip Kılavuzu. 1999. [cited Jully 2016]. Avaliable from: http://www.tkd.org.tr.
3. Medenica M, Ivanovic D, Maskovic M, Jancic B and Malenovic A. Evaluation of impurities level of perindopril tert-butylamine in tablets. J. Pharm. Biomed. Anal. 2007;44:1087–1094. https://doi.org/10.1016/j.jpba.2007.05.008
4. Simoncic Z, Roskar R, Gartner A, Kogej K ve Kmetec V. The use of microcalorimetry and HPLC for the determination of degradation kinetics and thermodynamic parameters of Perindopril Erbumine in aqueous solutions. Int. J. Pharm. 2008;356(1-2):200–205. http://dx.doi.org/10.1016/j.ijpharm.2008.01.031
5. El-Gizawy SM, Abdelmageed OH, Deryea SM, Omar MA and Abdel-Megied AM. Chiral separation of perindopril erbumine enantiomers using high performance liquid chromatography and capillary electrophoresis. Anal. Methods. 2014;6(3):825–830. http://dx.doi.org/10.1039/c3ay42056f
6. Maskovic M, Dotsikas Y, Malenovic A, Jancic-Stojanovic B, Ivanovic D and Medenica M. Desirability-based optimization and its sensitivity analysis for the perindopril and its impurities analysis in a microemulsion LC system. Microchem. J. 2011;99(2): 454–460. https://doi.org/10.1016/j.microc.2011.06.022
7. Katalin KE, Kelemen LJ, Gyeresi A, Silvia I and Mona O. Screening the Dissolution Performance of the Modified Release Tablets Containing Insoluble Active Substance in Different Dissolution Media. Acta Med Marisiensi. 2012;58(6):401-404.
8. Legorburu MJ, Alonso RM, Jiménez RM and Ortiz E. Quantitative Determination of Indapamide in Pharmaceuticals and Urine by High Performance Liquid Chromatography with Amperometric Detection. J. Chromatogr. Sci. 1999;37(8):283-287. https://doi.org/10.1093/chromsci/37.8.283
9. Kumar SA, Debnath M, Seshagiri Rao JVLN and Sankar DG. A new and rapid analytical method development & validation for simultaneous estimation of hydrochlorothiazide, amlodipine & olmesartan in tablet dosage form by using RP-HPLC. J. Chem. Pharm. Res. 2014;6(5):1208-1213.
10. Patel J and Patel M. RP-HPLC method development and validation for the simultaneous estimation of ramipril and amlodipine besylate in capsule dosage form. J. Chem. Pharm. Res. 2014;6(6):725-733.
11. Ubale M. RP-HPLC Method for Simultaneous Determination of Amlodipine Besylate and Atorvastatin Calcium: Applicati-

ISSN: 2458 - 8806

on to Commercially available drug products. Int. Arch. App. Sci. Technol. 2014; 5(1):22-27.

12. Elshanawane AA, Abdelaziz LM, Kamal MM and Hafez HM. Quantitative Determination of Telmisartan, Ramipril, Amlodipine Besylate and Atorvastatin Calcium by HPLC. J. Liq. Chromatogr. R.T. 2014;37(2):195–206. https://doi.org/10.1080/10826076.2012.738622
13. Peikova LP, Tsvetkova BG, Kostova BD and Rache DR. Investigations and HPLC Assay of Model Formulations Containing Amlodipine Besylate and Lisinopril. Int. J. Pharm. Sci. Rev. Res. 2013;20:11-15.
14. Alagar RM, Ragavendra P, Banji D, Rao KNV, Chaithanya Y, Anusha B and Selvakumar D. RP-HPLC Method for Simultaneous Estimation of Aliskiren Hemi Fumarate, Hydrochlorothiazide and Amlodipine in Pharmaceutical Bulk Drugs and Tablet Dosage Form. J. Pharm. Res. 2012;5(8):4580-4584.
15. Shaalan RA, Belal TS, El Yazbi FA and Elonsy SM. Validated Stability-İndicating HPLC-DAD Method of Analysis for The Antihypertensive Triple Mixture of Amlodipin Besylate, Valsartan and Hydrochlorothiazide in Their Tablets. Arab. J. Chem. 2017;10:S1381-S1394. https://doi.org/10.1016/j.arabjc.2013.04.012
16. Nalluri BN, Naik DV, Sunandana B, Sushmith K. Development and validation of RP-HPLC-PDA method for the simultaneous estimation of hydrochlorothiazide, amlodipine besylate and olmesartan medoxomil in bulk and pharmaceutical dosage forms. J. Chem. Pharm. Res. 2013;5(1):329-335.
17. Sinojiya RS, Khasia V, Patel BJ, Dangi AA. Development and Validation of Rp-HPLC Method for the Simultaneous Determination of Telmisartan, Amlodipine Besylate & Hydrochlorothiazide in a Tablet Dosage Form. J. Pharm. Res. 2012 5(8):4154-4157.
18. Kochar NI, Dahake MN, Bakal RL, Devani AP, Chandewar AV. Method Development and Validation of Ramipril and Amlodipine Besylate by RP-HPLC. Asian J. Sci. Res. 2011; 4(12):1829-1832.
19. Tamboli AM, Chavan C, Mohite SK. Development and Validation of a RP- HPLC method for simultaneous determination of Amlodipine Besylate and Enalapril Maleate. J. Pharm. Res. 2010;3(11):2564-2567.
20. Khomushku GM, Zhloba AA, Puchnin VS, Arkhipova MV, Moiseeva SM. Analyses of the Angiotensin-Converting Enzyme Inhibitors Perindopril, Lisinopril and Quinapril by HPLC. Pharm Chem J. 2011;44(11):619-623. https://doi.org/10.1007/s11094-011-0531-0
21. El-Gindy A, Nassar MW, Abdel-Salam Attia K, Abu-Seada H, El-Ghandour M. Stability-Indicating HPLC Method for Simultaneous Determination of Captopril, İndapamide and Their Related Compounds. J. Liq. Chromatogr. R.T. 2014;37(5):696–712. https://doi.org/10.1080/10826076.2012.758138
22. Parusu T, Ponneri V. RP-HPLC method for simultaneous determination of atenolol and indapamide in pharmaceutical dosage forms, human blood and milk. Eur. J. Chem. 2012;3(2):138-142. https://doi.org/10.5155/eurjchem.3.2.138-142.537
23. Rani GT, Sankar DG, Kadgapathi P, Satyanarayana B. A Validated RP-HPLC method for simultaneous estimation of atenolol and indapamide in pharmaceutical formulations. Eur. J. Chem. 2011;8(3):1238-1245. https://doi.org/10.1155/2011/121420
24. Kadian N, Maste M, Bhat AR. An Effective RP-HPLC Method for the Simultaneous Determination of Atenolol and Indapamide in Marketed Tablet Formulation. Asian J. Chem. 2012;5(3):405-408.
25. Munot NM, Ghante MR, Deshpande AS, Gaikwad PV. Development and validation of RP-HPLC Method For Simultaneous Estimation of Indapamide and Telmisartan. J. Pharm. Res. 2010;3(12):2941-2943.
26. Basu A, Das B, Basak K, Chakraborty M, Basu S. Development & Validation of Stability Indicating High Performance Liquid Chromatographic Method for Simultaneous Estimation of Atenolol & Indapamide in Tablet Dosage Form. J. Pharm. Res. 2011;4(6):1677-1680.
27. Baheti KG, Shah N, Shaikh S. Ion-Pairing Reverse-Phase High Performance Liquid Chromatography Method for Simultaneous Estimation of Atenolol and Indapamide in Bulk and Combined Dosage Form. Indian J. Pharm. Sci. 2012; 74(3), 271–274.
28. Zaazaa HE, Abbas SS, Essam HAM and El-Bardicy MG. Validated chromatographic methods for determination of perindopril and amlodipine in pharmaceutical formulation in the presence of their degradation product. J. Chromatogr. Sci. 2013;51:533–543. https://doi.org/10.1093/chromsci/bms171
29. 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(5):717-724. https://doi.org/10.2478/s11532-013-0201-z
30. Gizawy SM, Bebawy LI, Abdelmageed OH, Omar MA, Deryea SM, Abdel-Megied AM. High performance liquid chromatography, TLC-densitometry and first derivative spectrophotometry for simultaneous determination of amlodipine and perindopril in bulk powder and its tablets. J. Liq. Chromatogr. R.T. 2013;36(10):1323-1339. https://doi.org/10.1080/10826076.2012.686141
31. Gumustas M. 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(4):751-757. https://doi.org/10.5740/jaoacint.11-010

32. Prajapati J, Patel A, Patel MB, Prajapati N, Prajapati R. Analytical method development and validation of Amlodipine besylate and Perindopril erbumine in combine dosage form by RP-HPLC. Int. J. Pharm. Sci. Res. 2011;3(2):801-808.
33. Jain PS, Badreshiya PR, Chalıkwar SS, Todarwal AA, Surana SJ. Validation of a dissolution method with RP-HPLC analysis for perindopril erbumin and indapamide combination tablet. Chem. Ind. Chem. Eng. Q. 2012;18(1):19−25. https://doi.org/10.2298/CICEQ110628042J
34. Pathak AK, Lodhi R, Shikhri MK. A Selective Reverse Phase HPLC Method For Simultaneous Determination of Perindopril and Indapamide In Solid Dosage Form And Bulk Drug. J. Pharm. Res. 2011; 4(8), 2512-2513.
35. Erk N. Comparison of spectrophotometric and an LC method for the determination perindopril and indapamide in pharmaceutical formulations. J. Pharm. Biomed. Anal. 2001;26:43-52. https://doi.org/10.1016/S0731-7085(01)00388-0
36. Bhadani S, Sellappan M. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Perindopril Erbumine and İndapamide in Combined Dosage Form. Am. J. Pharm. Educ. 2013; 3(3), 703-711.
37. Jogia H, Khandelwal U, Gandhi T, Singh S, Modi D. Development and validation of a stability-indicating assay method for simultaneous determination of perindopril and indapamide in combined dosage form by reversed-phase high-performance liquid chromatography. J. AOAC Int. 2010;93(1):108-115. https://doi.org/10.1093/jaoac/93.1.108
38. Joseph J, Philip B, Sundarapandıan M. Method development and validation for simultaneous estimation of perindopril erbumine and indapamide by RP-HPLC in pharmaceutical dosage forms. Int. J. Pharm. Sci. 2011; 3(4).
39. Patel DB, Mehta FA, Bhatt KK. Simultaneous Estimation of Amlodipine Besylate and Indapamide in a Pharmaceutical Formulation by a High Performance Liquid Chromatographic (RP-HPLC) Method. Sci. Pharm. 2012;80:581–590. https://doi.org/10.3797/scipharm.1203-07
40. Mahajan SM, Chaple DR, Asnani AJ, Dhole SM. Development of RP-HPLC Method for Estimation of Indapamide and Amlodipine Besylate in Pharmaceutical Formulation. Asian J. Chem. 2012;5(6):738-741.
41. Kaila HO, Ambasana MA, Shah AK. Development and Validation of a Reversed-Phase Ultra-Performance Liquid Chromatographic Method for the Simultaneous Determination of Six Drugs Used for Combined Hypertension Therapy. J. AOAC Int. 2013;96(2):295-300. https://doi.org/10.5740/jaoacint.10-466
42. ICH. (2023). *Validation of Analytical Procedures* Q2(R2). Harmonized Tripartite Guideline.
43. The United States Pharmacopeia. (2005). *Rockville: The national formulary,* United States Pharmacopeial Convention.
44. El-Bagary RI, Elkady EF, Mowaka S, Attallah MA. A Validated HPLC Method for Simultaneous Determination of Perindopril Arginine, Amlodipine, and Indapamide: Application in Bulk and in Different Pharmaceutical Dosage Forms. J. AOAC Int. 2017; 100:992–999. https://doi.org/10.5740/jaoacint.16-0279
45. Chaudhary BR and Dave JB. Estimation of Perindopril Arginine, Indapamide and Amlodipine in Bulk and Fixed Dose Combination Using Stability Indicating Reverse Phase High-Pressure Liquid Chromatography. Int. J. Pharm. Sci. Res. 2020;11(12): 6267-6278. https://doi.org/10.13040/IJPSR.0975-8232.11(12).6267-78
46. Metwally MB, Khater DF, Abu-Nameh ESM, AlRashdan Y, Qaisi AM and Salim M. Simultaneous Determination of Indapamide, Amlodipine Besylate and Perindopril Arginine Combined in Tablet Dosage Form Using High Performance Liquid Chromatography. Jordan J. Pharm. Sci. 2020;13(4).
47. Swamy GK, Kumar JMR and Rao JS. Development and Validation of a Novel Stability Indicating RPHPLC Method for Simultaneous Estimation of Perindopril, Indapamide and Amlodipine in Bulk and Combined Tablet Dosage Forms. World J. Pharm. Res. 2015;4(6):2440-56.
48. Patel KP, Chhalotiya UK, Kachhiya HM and Patel JK. A new RP–HPLC method for simultaneous quantification of perindopril erbumine, indapamide, and amlodipine besylate in bulk and pharmaceutical dosage form. Future J. Pharm. Sci. 2020;6:1-9. https://doi.org/10.1186/s43094-020-00092-4
49. Karadurmus L, Gumustas M, Kurbanoglu S, Uslu B, Ozkan SA. A Novel Core-Shell-Based Chromatographic Method Supported by Ratio Derivative Spectrophotometry for The Simultaneous Determination of Perindopril, Indapamide, and Amlodipine Ternary Mixtures. Turk. J. Chem. 2018;42:1408-1419. https://doi.org/10.3906/kim-1712-9

ISSN: 2458 - 8806