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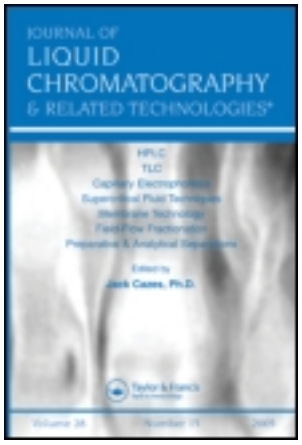

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY, TLC-DENSITOMETRY, AND FIRST-DERIVATIVE SPECTROPHOTOMETRY FOR SIMULTANEOUS DETERMINATION OF AMLODIPINE AND PERINDOPRIL IN BULK POWDER AND ITS TABLETS

Samia M. Gizawy [a], Loris I. Bebawy [b], Osama H. Abdelmageed [c], Mahmoud A. Omar [d], Sayed M. Deryea [d] & Ahmed M. Abdel-Megied [e]

[a] Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Assuit University, Assuit, Egypt

[b] National Organization for Drug Control and Research, Ben-El-Saraiat, Dokki, Gizza, Egypt

[c] Pharmaceutical Chemistry Department, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia

[d] Analytical Chemistry Department, Faculty of Pharmacy, Minia University, Minia, Egypt

[e] Analytical Chemistry Department, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), 6th October City, Egypt

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY, TLC-DENSITOMETRY, AND FIRST-DERIVATIVE SPECTROPHOTOMETRY FOR SIMULTANEOUS DETERMINATION OF AMLODIPINE AND PERINDOPRIL IN BULK POWDER AND ITS TABLETS

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[1]*Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Assuit University, Assuit, Egypt*
[2]*National Organization for Drug Control and Research, Ben-El-Saraiat, Dokki, Gizza, Egypt*
[3]*Pharmaceutical Chemistry Department, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia*
[4]*Analytical Chemistry Department, Faculty of Pharmacy, Minia University, Minia, Egypt*
[5]*Analytical Chemistry Department, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), 6th October City, Egypt*

□ *Three simple, sensitive, and specific methods were developed for simultaneous determination of amlodipine besylate (AML) and Perindopril Erbumine (PER) without previous separation. The first method was dependent on the first derivative of the ratio spectra by measuring the amplitudes at 348 nm for amlodipine using 50 μg mL^{-1} of perindopril as a divisor and at 227 nm for perindopril using 30 μg mL^{-1} of amlodipine as a divisor. The second method was based on ion-pair RP-HPLC. Satisfactory resolution was achieved using RP-C18 chromatographic column Zorbax Extend column and a mobile phase consists of potassium dihydrogen phosphate buffer (0.05 M, pH 3.0± 0.02 adjusted by orthophosphoric acid): acetonitrile 30:70 v/v at a flow rate 1 mL/min using 0.002 M sodium heptanesulfonate in the aqueous phase. UV detection was performed at 215 nm. The third method was based on TLC; the separation was carried out on Fluka TLC aluminum sheets silica gel 60 F_{254}, using n-butanol:water:glacial acetic acid (4:5:1, v/v/v) as the mobile phase. The validation of the proposed methods was applied according to ICH guidelines and LOD and LOQ were calculated. The suggested methods were successfully applied for the determination of the cited drugs in bulk powder and commercial tablets.*

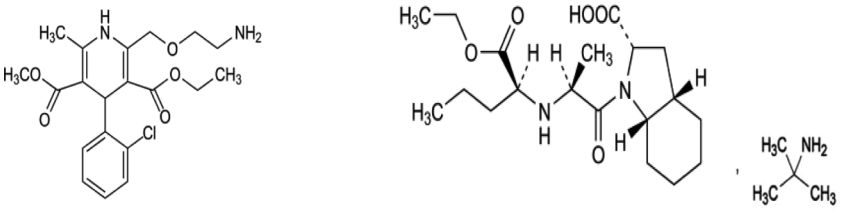
Address correspondence to Dr. Ahmed Mohamed Abdel-Megied, PhD, October University for Modern Sciences and Arts (MSA), Pharmaceutical Analytical Chemistry, 26 July Mehwer Road Intersection with Wahat Road, 6th October City, Egypt, Gizah, 6th October, 61111 Egypt. E-mail: dr_ahmed80@hotmail.com

Keywords amlodipine besylate, COVERAM tablets, derivative ratio spectrophotometry, perindopril erbumine, RP-HPLC, TLS-Densitometry

INTRODUCTION

Amlodipine besylate (AML), 2-[(2-amino ethoxy)-methyl]-4-(2-cholophenyl)-1,4-dihydro-6-methyl-3,5-pyridine dicarboxylic acid 3-ethyl-5-methyl ester, benzosulfonate, is a potent dihydropyridine calcium channel blocker used as an antihypertensive agent. Similar to other calcium channel blockers, amlodipine acts by relaxing the smooth muscle in the arterial wall, decreasing peripheral resistance, and hence reducing blood pressure. In angina, it increases blood flow to the heart muscle.[1] Perindopril Erbumine (PER), (2S,3aS,7aS)-1-[(2S)-2-[[(1S)-1(Ethoxycarbonyl)butyl] amino]-1–oxopropyl]octahydro–1H-indole–2–carboxylic acid compound with tert-butylamine (1:1), belongs to a group called angiotensin converting enzyme (ACEIs) which is used as antihypertensive agents.[2] Recently, perindopril has been marketed in combination with amlodipine in tablets for the treatment of hypertension.[3] This combination is advised for patients whose blood pressure is not adequately controlled by either drug alone. A literature survey revealed that a number of methods have been reported for estimation of AML and PER individually or in combination with other drugs.[4,5] The reported methods are UV spectrophotometry,[6–12] spectrofluorimetry,[13] HPLC,[4,14–21] HPTLC,[22–25] capillary electrophoresis,[26–28] and electrochemical methods.[29–32] However, there are only two analytical methods reported for the simultaneous analysis of both drugs in combined dosage formulation.[4,5] The structure of the studied drugs is shown in the following Scheme 1:

The aim of this work was the development of simple, sensitive, and accurate analytical methods for the simultaneous determination of amlodipine besylate and Perindopril Erbumine in mixtures without the need of prior separation step. The developed methods to determine the content of the cited drugs in commercial tablets was also demonstrated.



SCHEME 1 Amlodipine besylate (AML) Perindopril Erbumine (PER).

EXPERIMENTAL

Apparatus

A UV-Visible Spectrophotometer, Shimadzu UV-1800 (Shimadzu, Japan), connected to an IBM compatible computer was utilized. The software was UVPC personal spectroscopy software version 3.7 (Shimadzu). The absorption spectra of the reference and test solutions were carried out in a 1-cm quartz cells.

Agilent 1200 series HPLC (Agilent, Germany) was comprised of a vacuum degasser, an auto sampler, a thermostated column compartment G1316A/G1316B, a multiple wavelength detector SL, and a quatenary pump (Germany). A chromatographic column, Zorbax ODS (4.6 cm × 150 mm, 5 μm), P.N. 880952702, S.N. USF 0060920 was utilized in this work. Chromatographic peaks were electronically integrated and recorded using Chemstation software (Agilent Chemstation V. B.03.01, Germany).

The TLC aluminum-backed precoated plates were silica gel 60 F_{254}, layer thickness 0.25 mm, 20 × 20 cm (Fluka, Buchs, Switzerland).

A TLC scanner 3D densitometer Model 3 S/N 130319 was connected with winCats software (Camag, Muttenz, Switzerland).

The instrumental parameters are taken into consideration as follows:

- Slit dimensions: 6.0 × 0.45 mm
- Scanning speed: 20 mm/s
- Spraying rate: 10 s/mL
- Data resolution: 100 mm/step
- Band width: 6 mm
- Result output: Chromatogram and integrated peak area

A sample applicator for TLC Linomat IV with a 100-μL syringe (Camag, Muttenz, Switzerland) was utilized for this work. Also, an ultrasonic bath by Bandelin Songrex (Sigma-Aldrich, St. Louis, MO) and a Jenway 3305 pH/mv Meter with double junction glass electrode (Fisher, USA) were also employed in this work.

Materials and Reagents

Amlodipine byselate was kindly supplied as gift samples by Pfizer Egypt S.A.E. (Cairo, Egypt); perindopril erbumine was kindly provided by ADWIA Pharmaceuticals Co. (El-Oubor city, 1st Industrial Area, Cairo, Egypt), certified to contain 99.7% *w*/*w* and 99.2% *w*/*w* on dried basis for AML and PER, respectively.

COVERAM (B. No. 91222) was manufactured by Servier (Ireland) Industries Ltd. for les Laboratories Servier-France, Packed by Servier Egypt

Industries Limited, (6th October City, Egypt) and labeled to contain 5 mg AML and 5 mg PER for each tablet.

Sodium heptanesulfonate was provided by Sigma-Aldrich, Chemie GmbH (Taufkirchen, Germany). Acetonitril of HPLC grade was provided by Prolabo (Paris, France). Methanol of HPLC grade was provided by Fisher Scientific (Loughborough, UK). Potassium dihydrogen phosphate and orthophosphoric acid were of HPLC grade and provided by Merck (Darmstadt, Germany). Double distilled water used in preparation of the mobile phase. It was prepared by double glass distillation and filtrated through a 0.45-μm membrane filter. Glacial acetic acid, n-butanol was obtained from BDH chemicals (Poole, England) and was of analytical grade.

Chromatographic Conditions

Ion-Pair RP-HPLC Method

The mobile phase was prepared by mixing potassium dihydrogen phosphate buffer (0.05 M, pH 3.0 ± 0.02 adjusted by orthophosphoric acid): acetonitrile 30:70, *v*/*v* using 0.002 M sodium heptanesulfonate in the aqueous phase. The mobile phase was filtered using 0.45-μm membrane filter (Millipore, Milford, MA) and degassed by ultrasonic vibration prior to use. The samples were also filtered through 0.45-μm disposable filters. The flow rate was 1 mL/min; the column temperature was maintained at 50°C and detection was performed at 215 nm for all determinations.

TLC-Densitometric Method

The plates were developed in n-butanol:water:glacial acetic acid (40:50:10 by volume) after shaking and using the upper layer as a mobile phase. For detection and quantitation, 10 μL of test and 1.0-20.0 μL of standard solution were applied as separate bands of 6 mm width using a Camag Linomat IV applicator. Space the bands 5 mm from each other and 20 mm apart from the bottom edge of the plate of the plate. The plate was developed up to the top (over a distance 16 cm) in the usual ascending way. The chromatographic chamber was saturated with the mobile phase in the usual mode for 30 min after elution the plate was air dried and scanned at 365 nm and 215 nm for AML and PER, respectively, as under the described instrumental parameters.

Stock Solutions

All standard stock solutions were prepared freshly.

First Derivative Ratio Method (^{1}DD)

Standard stock solutions of AML and PER were prepared separately by dissolving 25 mg of each compound in 25-mL volumetric flasks (1.0 mg mL^{-1}) using methanol. Aliquots of 0.1 to 0.6 mL AML and 0.2 to 0.8 mL PER were transferred into 10-mL volumetric flasks to reach the concentration ranges of 10–60 μg mL^{-1} for AML and 20–80 μg mL^{-1} for PER.

Ion-Pair RP-HPLC Method

Primary stock solutions of AML and PER were prepared daily separately by dissolving 25 mg of each in 25 mL volumetric flasks (1.0 mg mL^{-1}) in mobile phase. Series of working solutions of AML and PER were prepared by the appropriate dilution of the stock solutions with same solvent to reach the concentration ranges of 5–200 μg mL^{-1} for AML and 5–120 μg mL^{-1} for PER.

TLC-Densitometric Method

Standard stock solutions of AML prepared by dissolving 25 mg of each in 25-mL volumetric flasks (1.0 mg mL^{-1}) in methanol and PER were prepared daily separately by dissolving 50 mg of each in 25-mL volumetric flasks (2.0 mg mL^{-1}) in methanol.

Procedures

First Derivative Ratio Method (^{1}DD)

The standard solutions were prepared by dilution of the stock standard solution with methanol to reach a concentration range 10.0–60.0 μg mL^{-1} for AML and 20.0–80.0 μg mL^{-1} for PER. Absorption spectra were recorded for each solution and stored in the IBM-Pc. For AML each of the absorption spectrum was recorded and divided by the spectrum of PER with the concentration 50.0 μg mL^{-1} as a divisor. The first derivative of the ratio spectra were calculated with $\Delta\lambda = 5$ nm, AML can be determined by measuring the first derivative amplitude of the ratio spectra at 348 nm. For PER, similar procedure was followed using 30.0 μg mL^{-1} AML as a divisor. PER was determined by measuring the amplitude value of the ratio spectra at 227 nm.

The calibration graphs were plotted representative the concentration values of ^{1}DD amplitude and the regression equations were computed.

Ion-Pair RP-HPLC Method

The standard solutions were prepared by dilution of the stock standard solution with mobile phase to reach a concentration range 5.0–200.0 μg mL^{-1} for AML and 5.0–120.0 μg mL^{-1} for PER. Triplicate 20.0 μL

injections were made for each concentration and chromatographed under the aforementioned condition. The peak area of each concentration was plotted against the corresponding concentration to obtain the calibration graph and the regression equation was computed.

TLC-Densitometric Method

The standard solution from 1.0–6.0 µL and 1.0–20.0 µL of stock standard solution of AML and PER, respectively, were applied to the TLC plates. Triplicate applications were made for each solution. The plate was developed as under the chromatographic conditions. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs and the regression equations were computed.

Sample Preparation

Twenty Coveram tablets were weighed and finely powdered. A portion of the powder equivalent to 50 mg of AML and PER was weighed accurately, dissolved in and diluted to 50 mL with methanol for ratio derivatives and TLC methods.

The same amount of powder was taken and dissolved in a 50-mL volumetric flask with the mobile phase for the HPLC method. The sample solution was then filtered using 0.45-µm filters (Millipore, Milford, MA). The procedures were completed as previously mentioned and the concentrations of AML and PER were obtained from corresponding regression equations.

RESULTS AND DISCUSSION

First Derivative Ratio Method (^{1}DD)

As shown in Figure 1, zero-order spectra of standard drugs were found to be overlapped which made them difficult for simultaneous determination; the ratio derivative spectrophotometric method permits the determination of each component in their mixture at the wavelengths corresponding to a maximum or minimum. The main advantage of the ^{1}DD method is the chance of easy measurements in correspondence with peaks that permits the use of the wavelength of highest value of analytical signals (maximum or minimum). Moreover, the presence of a lot of maxima and minima is another advantage by the fact that these wavelengths gave an opportunity for the simultaneous determination of active compounds in the presence of excipients which possibly interfere with the analysis. The influence of $\Delta\lambda$ for obtaining the first derivative of the ratio spectra, as well as the effect of divisor concentration, was studied in order to select the best factors affecting

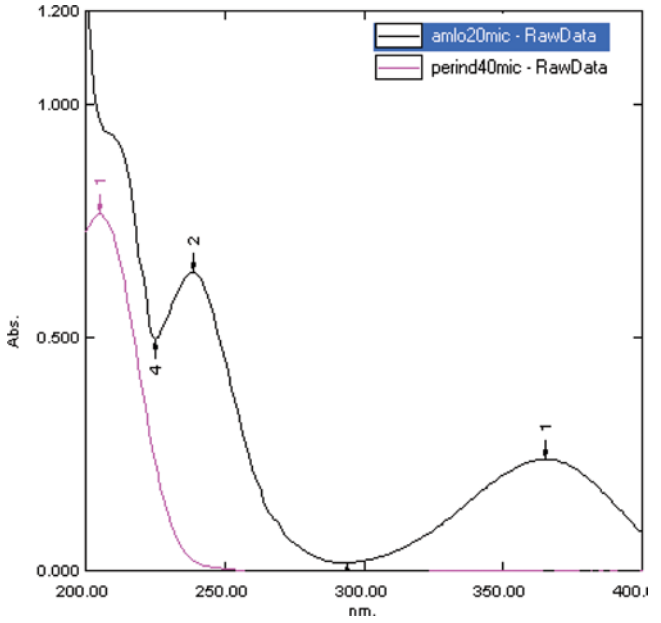


FIGURE 1 Zero-order absorption spectra of AML (—) and PER (—) ($30\,\mu g\,mL^{-1}$) for each in methanol. (Color figure available online.)

the determination. Results indicated that $\Delta\lambda = 5$ nm was the most suitable one. Determination of both drugs was accomplished by dividing the absorption spectra of AML by that of standard solution of PER ($50.0\,\mu g\,mL^{-1}$) while the absorption spectra of PER were divided by that of standard solution of

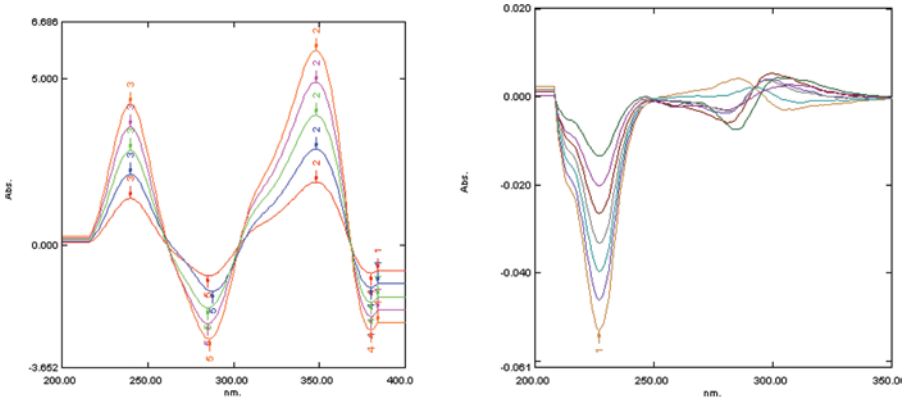


FIGURE 2 A) First derivative of the ratio spectra of AML (10–60 μg/mL) using $50\,\mu g\,mL^{-1}$ of PER as divisor. B) First derivative of the ratio spectra of PER (20–80 μg/mL) using $30\,\mu g\,mL^{-1}$ of PER as divisor. (Color figure available online.)

TABLE 1 Selected Spectral Data for the Determination of Cited Drugs by the Proposed Methods

Parameters	Perindopril			Amlodipine		
	^{1}DD	HPLC	TLC	^{1}DD at $\lambda_{max} = 348$	HPLC	TLC*
Linearity range μg mL^{-1}	20–80	5–120	2–10	10–60	5–200	1–6
Mean ± RSD	99.0 ± 0.39	99.7 ± 0.35	99.4 ± 0.89	98.0 ± 1.07	97.8 ± 1.34	98.7 ± 0.99
Intercept (a)	−0.001	−23.92	3967.7	−0.106	104.08	11808
SE of intercept (S_a)	−0.0004	8.88	78.79	0.034	44.27	441.85
Slope (b)	−0.0006	22.19	1046.7	0.094	31.52	5109.2
SE of slope (S_b)	0.0008	0.154	12.69	0.0008	0.46	113.46
Correlation Coefficient (r)	0.9996	0.9997	0.9994	0.9998	0.9994	0.9991
Determination coeff. (r^2)	0.9993	0.9993	0.9989	0.9997	0.9987	0.9983
SD of residuals ($S_{y.x}$)	0.0004	15.93	115.35	0.037	79.65	474.62
LOD (μg mL^{-1})	2.20	1.38	0.24	1.19	4.62	0.28
LOQ (μg mL^{-1})	6.66	4.01	0.752	3.61	14.01	0.86

*The concentration in TLC method is μg spot^{-1}.

AML ($30.0\,\mu g\,mL^{-1}$). The first derivative of the developed ratio spectra were calculated with $\Delta\lambda = 5$ nm. Figure 2A shows that AML can be determined by measuring the amplitude at 348 nm. PER can be determined by measuring the amplitude at 227 nm (Figure 2B). The proposed method is applicable over a concentration range of 10–60 μgmL^{-1} and 20–80 $\mu g\,mL^{-1}$ for AML and PER, respectively.

The characteristic parameters and necessary statistical data of the regression equation, limit of quantitation (LOQ), and limit of detection (LOD) are collected in Table 1.

Ion-Pair RP-HPLC Method

HPLC is considered the most powerful and versatile tool for quantitative determination of many individual components in a mixture in a single procedure.[33] The RP-HPLC method was developed to provide a specific procedure for the rapid quality control analysis of binary mixtures containing AML and PER. As shown in Figure 3, at a flow rate of 1 mL/min, the retention times for the investigated drugs were found to be 5.625 min and 11.135 min for AML and PER, respectively. To determine the appropriate HPLC conditions for separation of the examined drugs, various reversed phase columns, as well as isocratic and gradient mobile phase systems, were tried; successful attempts were performed using a RP-C18 chromatographic column Zorbax Extend column (150 mm × 4.6 mm i.d)

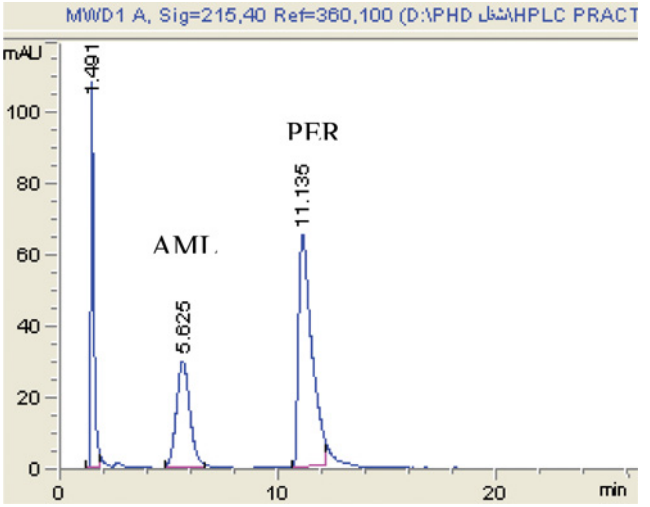


FIGURE 3 Representative HPLC chromatogram for a mixture of amlodipine ($100\,\mu g\,mL^{-1}$) and perindopril ($100\,\mu g\,mL^{-1}$) UV detection at 215 nm. (Color figure available online.)

5 µm particle size and a mobile phase consisted of mixture of potassium dihydrogen phosphate buffer (0.05 M, pH 3.0 ± 0.02 adjusted by orthophosphoric acid):acetonitrile 30:70, *v/v*. Addition of sodium heptane sulfonic acid sodium in the aqueous phase improved the separation. The column temperature was adjusted at 50°C for the highest resolution. The optimum wavelength for detection was 215 nm at which much better detection responses for the two drugs was achieved. Under the described HPLC parameters, the respective compounds were clearly separated and their corresponding peaks were sharply developed at reasonable retention times as shown in Figure 3. To determine the linearity of HPLC detection response, calibration standard solutions of AML and PER were prepared as described in the text. Linear correlation was obtained between the peak areas versus a concentration of each drug. The characteristic parameters for regression equations of the HPLC method are given in Table 1.

TLC-Densitometric Method

Experimental conditions such as mobile phase, band dimensions, scanning wavelength, and slit dimensions of scanning light beam were optimized to provide accurate, precise, and reproducible results for simultaneous determination of AML and PER. Initial experiments for the

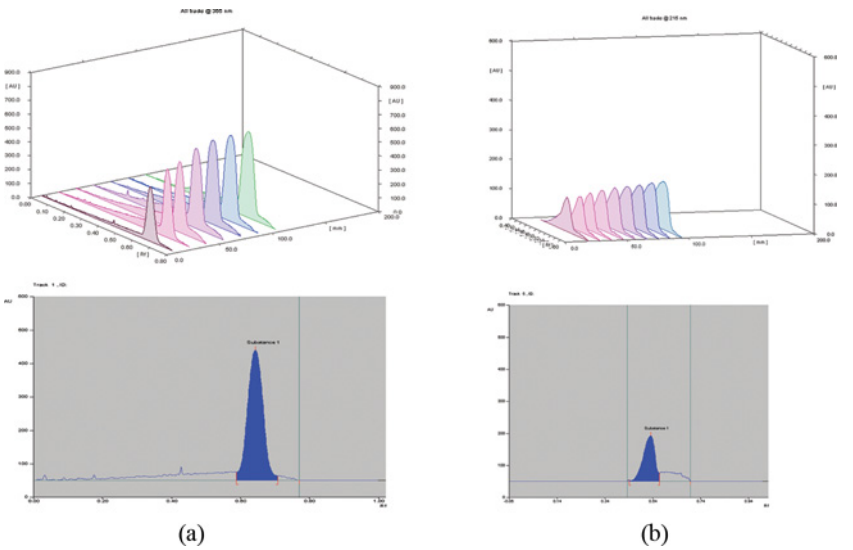


FIGURE 4 A) High Performance Thin layer Densitogram of amlodipine ($R_f = 0.72$) using n-butanol:-water:acetic acid in the ratio (4:5:1, *v/v/v*) as a developing system. B) High Performance Thin layer Densitogram of perindopril ($R_f = 0.48$) using n-butanol:water:acetic acid in the ratio (4:5:1, *v/v/v*) as a developing system. (Color figure available online.)

separation of AML and PER were performed using various proportions of different solvent systems, which resulted in varying retention factors, tailing of the peaks, larger run times, and moreover improper resolution of the components, as shown in Figure 4A,B.

The TLC method was very critical in this case for both drugs due to the opposite polarity (AML was highly polar and PER was nonpolar). The differences in R_f values of the two compounds (0.72 for AML and 0.48 for PER) with minimum tailing were obtained by using the mobile phase n-butanol:water:acetic acid in the ratio (4:5:1 by volume). The separated spots of the two drugs were scanned separately on the same plate at the specified wavelength 365 nm and 215 nm for AML and PER, respectively,

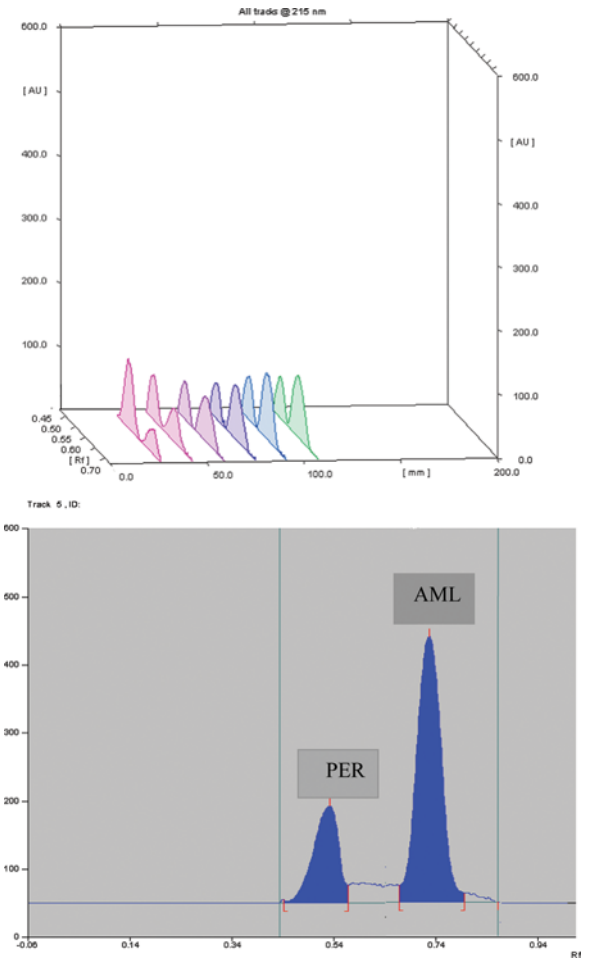


FIGURE 5 Representative High Performance Thin layer Densitogram of PER (5 μg spot $^{-1}$) and AML (10 μg spot $^{-1}$) in its tablet. (Color figure available online.)

as shown in Figure 5. The optimum band width chosen was 6 mm the interspaces between bands were sited 5 mm. Also, 5 mm × 0.2 mm proved to be the slit dimensions of choice which provide the highest sensitivity. The relationship between the concentration of each AML, PER, and the peak area of the spot was investigated. The calibration graphs were constructed in the range 1.0–6.0 and 2.0–10.0 μg spot^{-1} for AML and PER, respectively. The characteristic parameters of the regression equations of the two drugs are shown in Table 1.

SYSTEM SUITABILITY

According to U.S. Pharmacopeia (USP)[34] states that system suitability tests are an integral part of liquid chromatographic methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. For HPLC method, it was used to verify that the column efficiency (N), selectivity factor (resolution), and reproducibility of the chromatographic system. Parameters including resolution (Rs), peak symmetry, capacity factor (K$^{'}$), and selectivity factor (α) were calculated, as shown in Table 2.

METHOD VALIDATION

The suggested analytical procedures were subjected to validation according to ICH guidelines.[35,36] The developed methods were validated with respect to parameters such as linearity, precision, accuracy, and specificity, as shown in Table 3.

TABLE 2 Analytical Parameters for System Suitability Test of HPLC

		HPLC	
Parameter	Reference value	PER	AML
Flow rate (mL/min)	—	1.0	
Retention time (min)	—	5.50	11.1
Resolution (R)	R > 2	6.29	5.46
Selectivity factor (α)	>1	2.23	2.37
K' (Column capacity)	0.1–10 acceptable	2.76	6.44
Symmetry	—	0.85	0.42
Tailing Factor (T)	≤2	1.16	1.51
N (column efficiency)	Increases with efficiency of the separation	1446	1826
Height equivalent theoretical plates (HETP)	The smaller the value, the higher the column efficiency = L/N	0.033 cm	0.008 cm

TABLE 3 Evaluation of the Accuracy and Precision of the Proposed Three Methods

				DD1				HPLC				TLC			
Level				Taken $\mu g\,mL^{-1}$	Found* $\mu g\,mL^{-1}$	Recovery % (Mean ± SEM)	RSD (%) of Assay $n=3$*	Taken $\mu g\,mL^{-1}$	Found* $\mu g\,mL^{-1}$	Recovery % (Mean ± SEM)	RSD (%) of Assay $n=3$*	Taken $\mu g\ spot^{-1}$	Found* $\mu g\ spot^{-1}$	Recovery % (Mean ± SEM)	RSD (%) of Assay $n=3$*
PER	Interday	3	50%	20.0	20.20	101.0 ± 0.15	0.254	30.0	29.94	99.8 ± 0.35	0.428	3.0	2.99	99.6 ± 0.45	0.700
		3	100%	40.0	39.34	98.3 ± 0.35	0.613	60.0	59.89	99.8 ± 0.38	0.777	6.0	5.88	98.0 ± 0.55	1.109
		3	150%	80.0	80.76	100.9 ± 0.24	0.477	120.0	119.94	99.9 ± 0.55	1.101	9.0	8.96	99.6 ± 0.67	1.356
AML	Intraday	3	50%	10.0	10.16	101.6 ± 0.48	0.770	40.0	39.86	99.6 ± 0.31	0.626	2.0	1.97	98.5 ± 0.65	1.310
		3	100%	20.0	20.22	100.1 ± 0.61	1.217	80.0	79.54	99.4 ± 0.69	1.389	4.0	3.92	98.0 ± 0.46	0.932
		3	150%	40.0	40.54	101.3 ± 0.25	0.751	160.0	159.48	99.6 ± 0.31	0.642	6.0	7.97	99.6 ± 0.52	0.835

*Average of 3 determinations.

Concentration was measured in $\mu g\,mL^{-1}$ for the HPLC, spectrophotometric methods and in $\mu g\ spot^{-1}$ for the TLC method.

TABLE 4 Determination of AML and PER in Their Pharmaceutical Preparations (COVERAM Tablet) and Application of Standard Addition Technique

	DD1					HPLC					TLC				
COVERAM	Taken $\mu g\,mL^{-1}$	Found %[a] ± SD	Pure Added $\mu g\,mL^{-1}$	Rocovery %[b]	Mean ± SD	Taken $\mu g\,mL^{-1}$	Found[a] % ± SD	Pure Added $\mu g\,mL^{-1}$	Rocovery %[b]	Mean ± SD	Taken $\mu g\,mL^{-1}$	Found[a] % ± SD	Pure Added $\mu g\,mL^{-1}$	Rocovery %[b]	Mean ± SD
PER	20	99.6 ± 0.897	10	101.2	100.2 ± 0.951	50	100.8 ± 0.992	10	98.4	99.8 ± 0.873	2	97.6 ± 1.665	3	100.2	99.4 ± 0.566
			20	99.7				30	99.7				5	99.9	
			50	99.2				40	100.4				7	99.3	
			60	100.9				60	100.6				8	99.2	
AML	20	99.7 ± 1.137	10	99.6	99.8 ± 0.494	50	100.4 ± 0.792	20	99.3	100.4 ± 0.708	2	97.3 ± 1.503	1	99.6	99.2 ± 0.382
			20	100.3				40	100.7				2	99.4	
			30	99.8				50	100.6				3	98.9	
			40	99.9				70	100.8				4	98.8	

[a]Average of 5 determinations.
[b]Average of 3 determinations.

TABLE 5 Statistical Comparison of the Proposed Methods and Reported HPLC Method for Determination of AML and PER in Bulk Powder

	PER				AML			
Parameters	DD1	HPLC	TLC	Reported[4]	DD1	HPLC	TLC	Reported method[4]
Mean	99.1	99.7	99.4	99.6	98.0	97.8	100.2	98.7
SD	0.40	0.36	0.89	0.42	1.07	1.32	0.48	0.98
RSD (%)	0.39	0.35	0.89	0.42	1.09	1.34	0.47	0.99
N	6	6	6	6	6	6	6	6
variance	0.16	0.129	0.79	0.17	1.14	1.74	0.23	0.96
Student's *t* test (2.18)	1.92	1.47	1.58	—	1.60	1.37	1.31	—
F-value (4.88)	1.03	1.15	1.09	—	3.87	1.37	4.61	—

The values between parenthesis are the theoretical values of *t*- and *F*- at P = 0.05.

*Reported HPLC method[4]; using C8 column, Mobile phase; Buffer: Acetonitrile (65:35) and pH adjusted to 2.6 with dilute Ortho- Phosphoric Acid was delivered at the flow rate 1.0 mL/min. Detection was performed at 210 nm.

TABLET ANALYSIS

The proposed methods were successfully applied for the simultaneous determination of AML and PER in commercial tablets of Coveram. Five replicate determinations were made. Satisfactory results were obtained for both drugs and were in good agreement with the label claimed as shown in Table 4.

Moreover, to check the validity of the proposed methods, the standard addition technique was applied by adding AML and PER to the previously analyzed tablets; the results collected in Table 4 suggest that there is no interference from any excipients that are normally present in tablets. The results of determination of AML and PER in tablets obtained from the proposed methods were compared with reported the HPLC method.[4] Statistical comparison of the results was performed with regard to accuracy and precision using the Student's *t*-test and *F*-ratio at 95% confidence. The values reveal that there is no significant difference between the proposed and reported method with respect to accuracy and precision, as shown in Table 5.

Content uniformity testing: it was applied according to USP pharmacopeia[34] on ten tablets separately and results were found between 98.0–102% as the range of pharmacopeia between 85–115%.

CONCLUSIONS

The proposed methods were found to be sensitive, reproducible, and accurate in the analysis of amlodipine and perindopril in tablet form without the interference of excipients. The most striking feature of ratio derivative spectrophotometry is its simplicity and rapidity, and it is not time-consuming which is required for RP-HPLC and TLC-densitometry.

The RP-HPLC method was found to be more sensitive than TLC method, while the TLC method has the advantages of short scan time, large sample capacity, and use of minimal volume of solvent.

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