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A novel core-shell-based chromatographic method supported by ratio derivative spectrophotometry for the simultaneous determination of perindopril, indapamide, and amlodipine ternary mixtures

Leyla KARADURMUŞ[1,2], **Mehmet GÜMÜŞTAŞ**[3,4,*], **Sevinç KURBANOĞLU**[2], **Bengi USLU**[2], **Sibel A. ÖZKAN**[2]

[1]Department of Analytical Chemistry, Faculty of Pharmacy, Adıyaman University, Adıyaman, Turkey
[2]Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University, Ankara, Turkey
[3]Department of Chemistry, Faculty of Arts and Sciences, Hitit University, Çorum, Turkey
[4]Department of Forensic Toxicology, Institute of Forensic Sciences, Ankara University, Ankara, Turkey

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Abstract: In this work, ratio spectra of the first derivative spectrophotometric and liquid chromatographic methods have been described for the first time for the simultaneous determination of perindopril (PER), indapamide (IND), and amlodipine (AML) in dosage forms. For chromatographic separations several mobile phase compositions were tested for efficient separation with the use of a new column technology related to superficially porous particles. Optimum chromatographic separation was achieved using a Kinetex C18 column (150 × 4.6 mm I.D. 5 μm) at a flow rate of 1.5 mL min^{-1}. The separation was carried out at 30 °C and the diode array detector was adjusted to 215 nm. As a comparison, a spectrophotometric method depending on the first derivative of the ratio spectra was developed. The first derivative of the ratio amplitudes at 227.2 nm for PER, 269.4 nm for AML, 292.0 nm for IND were selected. The proposed methods were successfully applied for the simultaneous assay of the drug combination in pharmaceutical dosage forms and the methods were compared to each other in terms of Student t and F tests for the comparison of their accuracy and precision parameters.

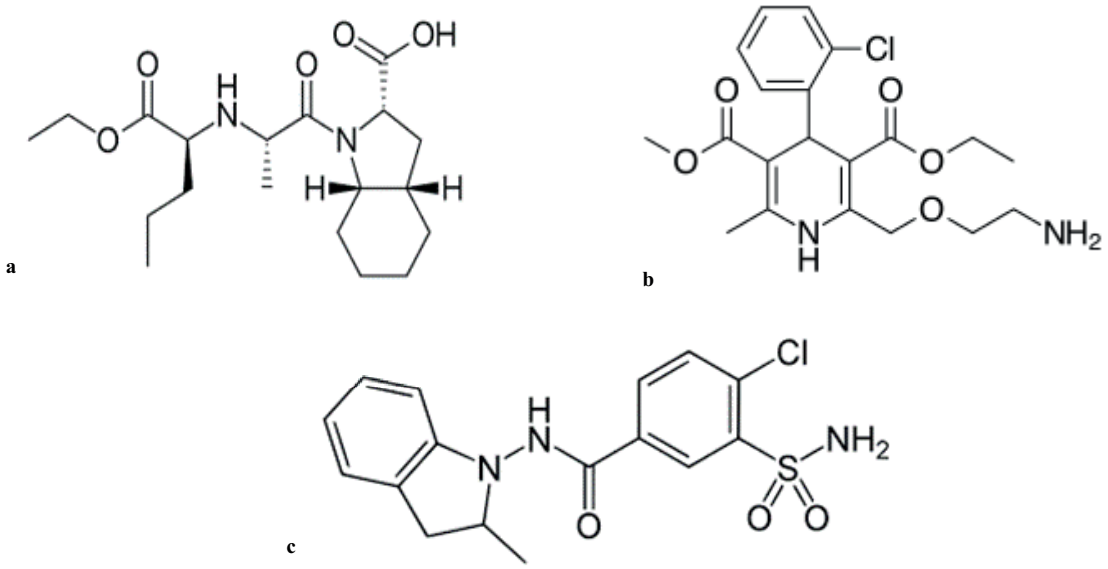
Key words: Amlodipine, indapamide, perindopril, ratio derivative spectrophotometry, high performance liquid chromatography

1. Introduction

Perindopril (PER) is an angiotensin converting enzyme (ACE) inhibitor used either alone or in combination in the treatment of hypertension. PER is essentially converted to perindoprilat, which is a prodrug with hydrolysis. PER is a moderate-action ACE inhibitor suitable for use in one or two daily doses. The drug blocks the conversion of angiotensin I to angiotensin II. PER is the free acid form of perindopril erbumine; in vivo, the biologically active metabolite is converted to perindoprilat by hydrolysis of the ester group. Unlike captopril, perindopril does not contain a sulfhydryl group, which is important for the profile of adverse effects.[1–4] Amlodipine (AML) is a member of the drug class of long-acting dihydropyridine calcium channel blockers used to treat hypertension and angina pectoris. AML, used in the treatment of hypertension, lowers the body's blood pressure by inhibiting calcium entry into the cell through calcium channels in the myocardium and smooth muscle cell membrane of calcium ions. AML is indicated for the treatment of hypertension and is used

*Correspondence: mgumustas@hotmail.com

either alone as monotherapy or in combination with other antihypertensive drugs[5−8] Indapamide (IND) is a benzamide-sulfonamide-indole derived diuretic and antihypertensive drug. Although the exact mechanism of action related to IND is unknown, it appears that the nephron is mainly effective in the distal tube fold. The drug inhibits sodium ion transport in kidney tubules, thereby increasing sodium, chloride, and water excretion[9−12] (Scheme).



Scheme. The structures of a) PER, b) AML, c) IND.

Combined drug therapy is based on the coadministration of two or more carefully selected drugs. The combination of PER/IND/AML is used for the treatment of arterial hypertension.[13−15] Initially, ACE inhibitors have been the target of treatment for hypertension and chronic heart failure. The use of ACE inhibitors in many diseases is due to their vasculoprotective, antiatherogenic, antiinflammatory, antiproliferative, and antithrombotic effects.[16−18]

Complex analytical methods require the use of preseparation processes such as derivatization and extraction in analytical processes. In the pharmaceutical industry, routine quality control laboratories and research laboratories use methods based on high performance liquid chromatography (HPLC) or hyphenated techniques in the analysis of mixtures containing more than one active pharmaceutical ingredient. The hyphenated techniques are particularly economically expensive and require skilled analysts, and the optimization of experimental conditions during analysis is tedious and time-consuming. It is clear that new analytical techniques and methods are needed to solve complex problems involving two or more active compounds and a sample matrix, which are economical, fast, and easy to apply and give accurate results.[19] In addition, new techniques in instrumentation with the help of column technology help to reduce or eliminate the disadvantages of classical and high-tech combined analytical methods in analytical procedures and to achieve more accurate and more precise analytical results compared to fully porous particles in conventional columns. In addition to this, it is necessary to select the temperature and pH stability of the packing material in order to obtain the most effective chromatographic separation. Some studies with superficially porous particles show that more effective analyses can be made in a shorter time when these materials are used.[20−25] Particle technology for liquid chromatography has been highly developed in recent years, and the use of core-shell particles has received great attention in its development. It

was observed that the solution flow in the columns containing fully porous silica particles was irregular and this irregularity led to tail spikes and peak expansions in peak shapes.[20−25] However, it has also been reported in the literature that efficient separations were achieved with stationary phases containing core-shell particles.[20−25] Core-shell particles have sharper peak shapes in a shorter time, rapid column conditioning after the changes in the mobile phase pH levels, relatively less solvent consumption, and more precise determination. More stable flow, more stable retention time of peaks, and precision between the results are achieved by superficially porous particles.[26−30] Thus, rapid analyses can be performed without loss of discrimination. Core-shell particles allow to reduce the mass transfer and to increase the peak efficiency. Along with the improvement in the peak efficiency, core-shell particle-based columns offer lower flow resistance in HPLC systems, higher resolution, and shorter analysis times, and sharper peak shapes may occur compared to the fully porous particles. In the literature, there exists binary determination of AML-PER, PER-IND, or AML-IND using HPLC.[29] Hence, no literature exists for the simultaneous detection of the ternary mixture using both new generation columns that have core-shell silica particles and ratio derivative spectrophotometry methods. The proposed study aimed to develop a more environmentally friendly, rapid, sensitive, and selective method for simultaneous determination of PER, IND, and AML using new generation HPLC columns. The accuracy and precision of the developed liquid chromatography method are compared with ratio derivative spectrophotometry by the means of Student t and F tests.

2. Results and discussion

2.1. Optimization of chromatographic conditions

Prior to the analyses the system was rinsed with the mobile phase. In order to separate PER, AML, and IND simultaneously, different kinds of columns and varied compositions of the mobile phases were tested. Mobile phase compositions were evaluated in terms of pH, acetonitrile/methanol/water ratios, and different flow rate values. Finally, optimum chromatographic separation was achieved using a Kinetex C18 column (150 $\times$ 4.6 mm I.D. 5 μm) at a flow rate 1.5 mL min^{-1} with temperature value of 30 °C and 215 nm. With these conditions, sharp peak shapes and precise retention time values were obtained. The system suitability tests were performed according to USP 40,[31] $< 621 >$, and are summarized in Table 1. It can be concluded from the parameters that the system was found suitable for the simultaneous analyses of PER, AML, and IND.

Table 1. System suitability tests parameters.

Technique	HPLC		
Compounds	PER	AML	IND
Retention time	1.33	1.8	2.6
Capacity factor (k)	0.5	1.02	1.92
Selectivity (α)	-	2.07	1.88
Resolution (Rs)	-	4.91	7.56
Tailing	1.09	1.25	0.986
Theoretical plate numbers (plate/column)	3436	5221	8438
RSD% of retention time	0.120	0.220	0.180

2.2. Determination and validation of AML, PER, and IND with DD_1 and chromatographic methods

In two or more compounds' analyses, the zero-order spectrum did not allow for simultaneous determination due to the considerable loss of accuracy and sensitivity by overlapping absorbance values. By deciding the wavelengths corresponding to a maximum or minimum of the ratio derivative spectrum, simultaneous determination can achieve two or more compounds. As shown in Figure 1A, absorption spectra of 4×10^{-4} M PER, 2×10^{-5} M AML, and 1×10^{-5} M IND in methanol can overlay and cannot achieve simultaneous determination. When ratio spectra are obtained, as shown in Figure 1B, for 4×10^{-4} M PER, divided by the mixture of 2×10^{-5} M IND and 4×10^{-5} M AML, acceptable results were obtained. Furthermore, 2×10^{-5} M AML (divisor is the mixture of 2×10^{-5} M IND and 8×10^{-5} M PER) and 1×10^{-5} M IND (divisor is the mixture of 4×10^{-5} M AML and 8×10^{-5} M PER) were also obtained.

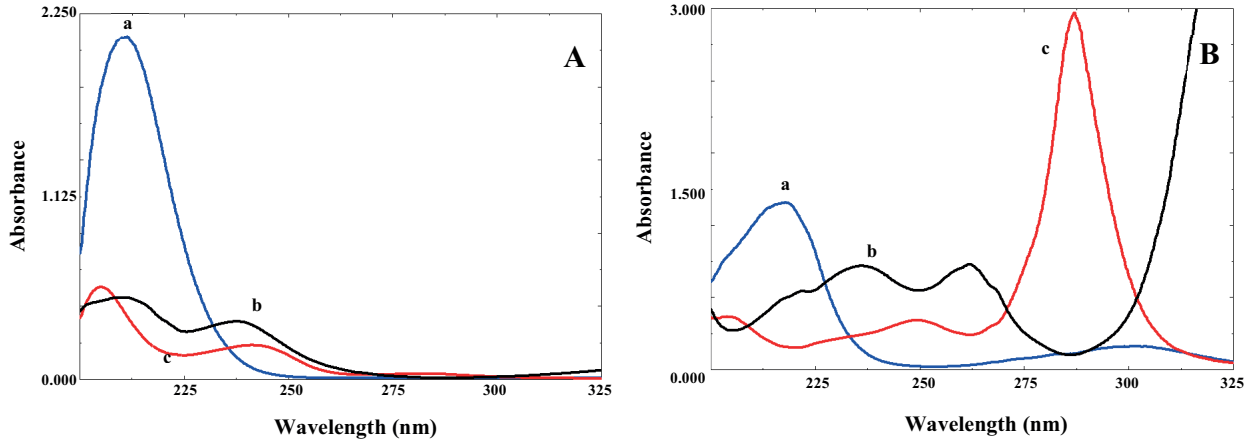


Figure 1. A) Absorption spectra of a) 4×10^{-4} M PER, b) 2×10^{-5} M AML, c) 1×10^{-5} M IND in methanol; B) Ratio spectra for a) 4×10^{-4} M PER, divisor 2×10^{-5} M IND + 4×10^{-5} M AML, b) 2×10^{-5} M AML, divisor 2×10^{-5} M IND + 8×10^{-5} M PER, c) 1×10^{-5} M IND, divisor 4×10^{-5} M AML + 8×10^{-5} M PER.

The linear ranges for the first derivative of the ratio spectra method were obtained between 2×10^{-5} M and 8×10^{-4} M for PER at 227.20 nm, 4×10^{-6} M and 6×10^{-5} M for AML at 269.40 nm, and 4×10^{-6} M and 2×10^{-5} M for IND at 292.00 nm. Using the suggested ratio derivative spectrophotometric method, PER, AML, and IND were successfully analyzed with LOD values of 4.78×10^{-7} M, 8.75×10^{-8} M, and 5.89×10^{-8} M, respectively (Figure 2A).

The chromatographic method was validated under optimized conditions using the new generation core-shell based Kinetex-C18 column (150 × 4.6 mm I.D. x 5 μm) at a flow rate of 1.5 mL min^{-1} at 30 °C and 215 nm. As shown in Figure 2B, retention times of PER, AML, and IND were obtained as 1.33 min, 1.80 min, and 2.60 min, respectively. Linearity ranges were obtained from 1.84×10^{-6} to 2.76×10^{-4} for PER, 1.76×10^{-6} to 2.11×10^{-4} for AML, and 2.73×10^{-6} to 2.73×10^{-4} for IND. Using this core-shell-based column, PER, AML, and IND were successfully analyzed with LOD values of 3.04×10^{-7} M, 1.55×10^{-7} M, and 1.66×10^{-7} M, respectively.

LOD, LOQ slope, intercept, standard error of slope, standard error of intercept, and correlation coefficient values were also calculated. Within-day precision and between-day precision results were given in terms of the percentage of relative standard deviation (RSD %). All the statistical evaluations are summarized in Table 2.

Table 2. Statistical evaluation of the calibration data by RP-LC and first derivative of the ratio spectra.

HPLC				UV-VIS		
	PER	AML	IND	PER	AML	IND
Retention time (min)/ wavelength (nm)	1.33	1.80	2.60	227.20	269.40	292.00
Linearity range (M)	1.84×10^{-6} to	1.76×10^{-6} to	2.73×10^{-6} to	2×10^{-5} to	4×10^{-6} to	4×10^{-6} to
	2.76×10^{-4}	2.11×10^{-4}	2.73×10^{-4}	8×10^{-4}	6×10^{-5}	2×10^{-5}
Slope	3×10^{6}	7×10^{6}	1.9×10^{7}	344.58	2663.90	22,029.00
Intercept	–0.133	5.079	–9.022	0.0004	0.0004	-0.0033
Correlation coefficient	0.999	0.999	0.999	0.999	0.999	0.999
SE of slope	3.4×10^{4}	4.6×10^{4}	1.8×10^{5}	2.68	25.2	66.8
SE of intercept	4.705	5.197	16.660	9.39×10^{-4}	7.27×10^{-4}	6.95×10^{-4}
Limit of detection (M)	3.04×10^{-7}	1.55×10^{-7}	1.66×10^{-7}	4.78×10^{-7}	8.75×10^{-8}	5.89×10^{-8}
Limit of quantification (M)	1.00×10^{-6}	5.11×10^{-7}	5.49×10^{-7}	1.45×10^{-6}	2.65×10^{-7}	1.79×10^{-7}
Within-day precision[a] (RSD%)*	0.116	0.071	0.040	0.216	0.178	0.437
Between-day precision[a] (RSD%)*	0.362	0.214	0.157	0.292	0.347	0.399

*Each value is the mean of five experiments.

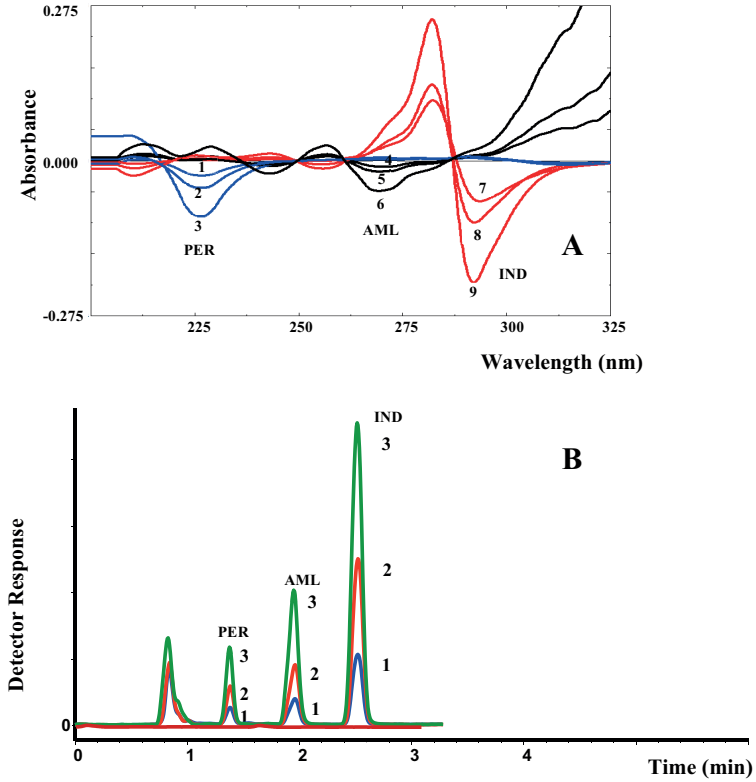


Figure 2. A) First derivative of the ratio spectra for 1) 1×10^{-4} M, 2) 2×10^{-4} M, 3) 4×10^{-4} M PER, divisor 2×10^{-5} M IND + 4×10^{-5} M AML; 4) 4×10^{-6} M, 5) 8×10^{-6} M, 6) 2×10^{-5} M AML, divisor 2×10^{-5} M IND + 8×10^{-5} M PER; 7) 2×10^{-6} M, 8) 5×10^{-6} M, 9) 1×10^{-5} M IND, divisor 4×10^{-5} M AML + 8×10^{-5} M PER. B) Chromatograms for increasing concentrations of 1) 3.68×10^{-6} M, 2) 9.20×10^{-6} M, 3) 1.84×10^{-5} M PER; 1) 3.52×10^{-6} M, 2) 8.80×10^{-6} M, 3) 1.76×10^{-5} M AML; and 1) 5.46×10^{-6} M, 2) 1.37×10^{-5} M, 3) 2.73×10^{-5} M IND.

2.3. Stability-indicating studies

Forced degradation studies were performed on standard AML, PER, and IND in order to produce the possible degradation products and their retention behavior by using developed HPLC methodology. For this reason, UV light (254 nm, 8 h), acid hydrolysis (0.1 M HCl), alkaline hydrolysis (0.1 M NaOH), oxidation (3% H_2O_2), heat in an oven (at 75 °C, 8 h), and heat in a water bath (at 75 °C, 2 h) stress conditions were applied to 9.20×10^{-5} M PER, 8.80×10^{-5} M AML, and 1.37×10^{-4} M IND. These experiments were conducted and the resultant peak areas were compared with the untreated constant amount of the standard solutions of each compound. It was aimed to show that PER, AML, and IND can be analyzed in the presence of their degradants and the specificity of the developed chromatographic method was adjusted as suggested in the International Council on Harmonization (ICH) guidelines (Figure 3). Results of stress conditions in terms of degradation amounts are summarized in Table 3 and calculations were carried out as indicated above.

2.4. Analysis of pharmaceutical dosage form

Coverdine tablets were analyzed, which contain 10.0 mg PER, 5.0 mg AML, and 2.5 mg IND in the presence of ingredients such as calcium carbonate, 10% pregelatinized maize starch, cellulose microcrystalline, croscarmellose sodium, magnesium stearate, colloidal anhydrous silica, pregelatinized starch, glycerol, hypromellose (6

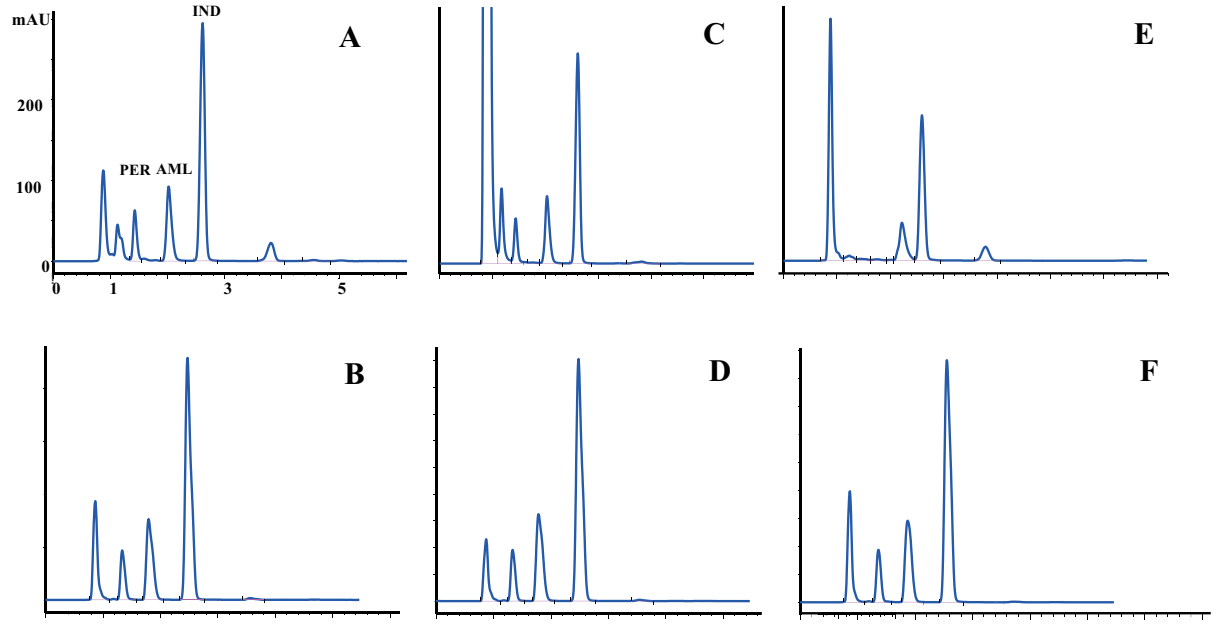


Figure 3. Stress degradation conditions for 9.20 × 10^{-5} M PER, 8.80 × 10^{-5} M AML, and 1.37 × 10^{-4} M IND: A) acidic hydrolysis (0.1 M HCl), B) oxidation (3% H_2O_2), C) alkaline hydrolysis (0.1 M NaOH), D) heating in oven (at 75 °C, 8 h), E) UV light (254 nm, 8 h), F) heating in water bath (at 75 °C, 2 h).

Table 3. Results of stress conditions by RP-LC in terms of degradation %.

Conditions	PER	AML	IND
HCl (0.1 M)	5.02	4.76	1.27
NaOH (0.1 M)	83.75	9.66	5.60
H_2O_2 (3%)	ND*	0.26	3.29
UV light exposure (8 h at 254 nm)	13.43	0.52	4.47
Oven (8 h at 75 °C)	12.7	5.02	4.55
Water bath (2 h at 70 °C)	14.59	5.83	6.07

*ND: No degradation.

mPa s), macrogol 6000, magnesium stearate, and titanium dioxide. With the proposed chromatographic and spectrophotometric methods, the Coverdine tablets were successfully analyzed in the presence of the indicated ingredients (Figure 4). One of the validation parameters, accuracy, was achieved by adding a known amount of pure drug active compound to Coverdine tablet solutions. For recovery studies reliable results were obtained, which are in between 96% and 103% recovery levels, as can be seen from Table 4.

2.5. Conclusions

In this work, a separation technique for AML, PER, and IND ternary mixture has been suggested for the first time based on a new generation column that has superficially porous particles used as a stationary phase in HPLC and the first derivative ratio spectrophotometric method. Both methods were further compared by using statistics with F and Student t tests. The results showed that there are no evocative differences between the methods statistically. Since the run time is only 5 min, the suggested method is rapid and can be used in

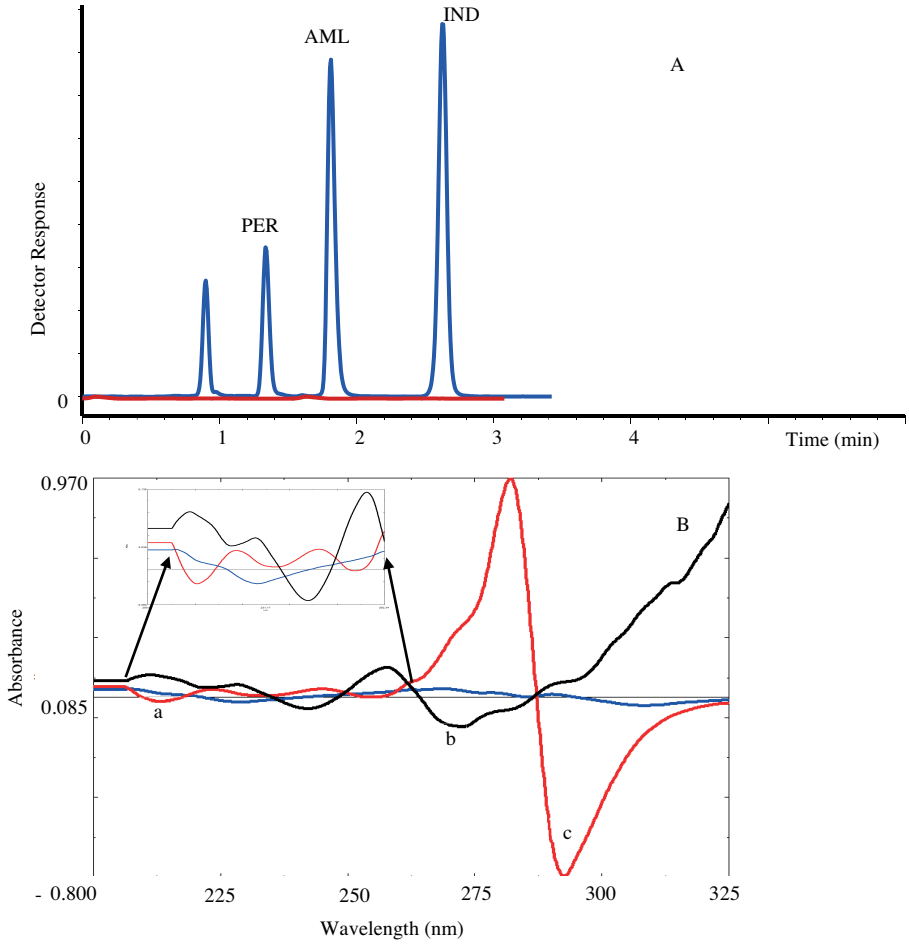


Figure 4. A) Chromatogram of pharmaceutical dosage form Coverdine. B) First derivative of the ratio spectra for pharmaceutical dosage form Coverdine: a) 7.92 × 10^{-5} M PER, divisor 2 × 10^{-5} M IND + 4 × 10^{-5} M AML, b) 2.57 × 10^{-5} M AML, divisor 2 × 10^{-5} M IND + 8 × 10^{-5} M PER, c) 2 × 10^{-5} M IND, divisor 4 × 10^{-5} M AML + 8 × 10^{-5} M PER.

research and development as well as in quality control laboratories where economy is essential. There are no sophisticated procedures such as extraction or additional cleanup procedures, resulting in an easy and applicable method. LOD values were obtained as 3.04 × 10^{-7} M, 1.55 × 10^{-7} M, and 1.66 × 10^{-7} M for PER, AML, and IND, respectively.

In conclusion, liquid chromatographic and spectrophotometric methods were developed and fully validated according to the ICH guidelines with respect to specificity, linearity, accuracy, and precision. The linearity ranges, LOD, and LOQ values were better than those of previously suggested methods, showing the power of the usage of superficially porous silica particles as a stationary phase in HPLC.[26–30]

3. Experimental

3.1. Materials and methods

For chromatographic studies, HPLC grade acetonitrile, methanol, orthophosphoric acid, sodium hydroxide, hydrogen peroxide, and hydrochloric acid were purchased from Sigma Aldrich (Munich, Germany). Methanol

Table 4. Results of analysis of pharmaceutical dosage forms by RP-LC and first derivative of the ratio spectra.

Technique	HPLC			UV-VIS		
Compounds	PER	AML	IND	PER	AML	IND
Labeled claim (M)	4.93×10^{-5}	4.83×10^{-5}	2.96×10^{-5}	4.93×10^{-5}	4.83×10^{-5}	2.96×10^{-5}
Amount found (M)	4.87×10^{-5}	4.66×10^{-5}	3.07×10^{-5}	4.97×10^{-5}	4.99×10^{-5}	2.98×10^{-5}
RSD (%)*	1.02	0.14	0.14	2.03	0.23	0.43
Bias (%)*	1.21	3.51	–4.02	–0.79	–3.73	–1.51
t value (t $_{Theo}$: 2.353)	0.066	0.024	0.039	-	-	-
F value (F $_{Theo}$: 9.280)	0.102	0.015	0.166	-	-	-
Added (M)	3.68×10^{-5}	3.52×10^{-5}	8.63×10^{-6}	3.0×10^{-5}	1.2×10^{-5}	1.0×10^{-5}
Found (M)*	3.69×10^{-5}	3.41×10^{-5}	8.61×10^{-6}	3.02×10^{-5}	1.20×10^{-5}	9.61×10^{-6}
Recovery (%)	100.45	97.03	99.76	100.60	99.60	96.06
RSD % of recovery*	0.34	0.15	0.10	0.81	0.26	1.36
Bias (%)	–0.45	2.96	0.23	–0.27	0.12	1.97

*Each value is the mean of five experiments.

(Merck, Darmstadt, Germany) and double distilled water were used for preparing the mobile phase solutions. PER and IND were from Nobel İlaç Sanayi A.Ş (İstanbul, Turkey), AML was from Sanovel İlaç Sanayi A.Ş (İstanbul, Turkey), and the commercial brand of ternary mixture Coverdine (PER/AML/IND, 10/5/2.5 mg, respectively) was obtained from Servier (Dublin, Ireland).

3.2. Equipment

The Agilent 1100 series LC system was used for chromatographic separations equipped with a G1379A degasser, G1311A quaternary pump, G1313 autoinjector, and G1315B diode array detector. Spectrophotometric experiments were conducted by Shimadzu 1601 PC double beam spectrophotometer equipped with 1.0 cm quartz cells with a fixed slit width (2 nm), coupled with a computer running Shimadzu UVPC software.

3.3. Chromatographic and spectrophotometric analyses conditions

The separation was carried out at 30 °C and the diode array detector was adjusted to 215 nm using the Kinetex C18 column (150 × 4.6 mm I.D. 5 μm) at a flow rate 1.5 mL min^{-1}. An isocratic mobile phase consisting of a mixture of acetonitrile and water (40:60, v/v) containing phosphoric acid (0.025%) was used for the simultaneous separation and analysis of active pharmaceutical ingredients. The spectrophotometric method depends on the first derivative of the ratio spectra by the division of the absorption spectrum of the ternary mixture by a standard spectrum of the binary mixture of the components and then calculating the first derivative of the ratio spectrum. The first derivative of the ratio amplitudes of the ratio spectra were recorded and the values of the derivatives were measured at suitably selected wavelengths in the range of 200 to 400 nm by plotting against the corresponding concentrations to obtain the calibration graph at 227.2 nm for PER, 269.4 nm for AML, and 292.0 nm for IND simultaneously. All solutions were protected from light, stored at 4 °C, and used within 24 h to avoid decomposition.

3.4. Preparation of solutions

Stock solutions of 1×10^{-3} M AML and IND were prepared in acetonitrile and PER was prepared in methanol. All solutions were then kept in dark at about 4 °C. Working solutions were prepared daily by the dilution of these stock solutions using the mobile phase and injected into the HPLC system. Standard solutions of 1×10^{-3} M AML, IND, and PER containing concentration ranges between 4×10^{-6} and 6×10^{-5} M, 4×10^{-6} and 2×10^{-5} M, and 2×10^{-5} and 8×10^{-4} M AML, IND, and PER were prepared in methanol, respectively.

3.5. Analysis of pharmaceutical dosage form

Ten tablets of Coverdine were accurately weighed, crushed, and finely powdered in a mortar. From this powder, the weight of the powder equivalent to one tablet's content was accurately weighed, transferred to a 100 mL calibrated flask, diluted with acetonitrile, sonicated about 10 min, and then completed to total volume with acetonitrile for HPLC study. For UV spectroscopy analysis, one tablet's content was accurately weighed, transferred to a 50 mL calibrated flask, diluted with methanol, sonicated about 10 min, and then completed to total volume with methanol. The pharmaceutical dosage form solutions were prepared related to a composition of solution that contained 10.0 mg, 5.0 mg, and 2.5 mg, PER, AML, and IND, respectively.

3.6. System suitability and validation of the method

According to ICH guidelines, the suitably of the system should be proven related to some parameters such as capacity factor (k), selectivity (α), resolution (R_s), tailing, and theoretical plate numbers (N).[32–35] After the system suitability was established based on the acceptability criteria, validation of the suggested methods was performed in terms of linearity, LOD and LOQ, precision, accuracy, etc. Linear ranges for the PER, AML, and IND were obtained separately, while limit of detection (LOD = 3.3 s/m) and limit of quantification (LOQ =10 s/m) were calculated using the standard deviation of response (s) and the slope (m) of the calibration curve. For the spectrophotometric method, the first derivative of the ratio spectra by division of the absorption spectrum of the ternary mixture by a standard spectrum of binary mixture of the components method (DD_1) was used. The first derivative of the ratio amplitudes at 227.2 nm for PER, 269.4 nm for AML, and 292.0 nm for IND were selected simultaneously.[31–35] Within the scope of the validation studies, recovery studies were also performed to demonstrate the applicability of the methods by adding a known pure amount of drug to the pharmaceutical dosage form. Recovery data were statistically compared with each method using the Student t and variance ratio F tests. In both tests, the calculated values did not exceed the theoretical values, which underlined that there were no significant differences between the recoveries from the HPLC and derivative-UV methods.

Moreover, degradation studies were accompanied with stress conditions as suggested in the ICH guidelines, such as UV light (254 nm, 8 h), acidic hydrolysis (0.1 M HCl), alkaline hydrolysis (0.1 M NaOH), oxidation (3% H_2O_2), and heat in an oven (at 75 °C, 8 h), to assess the stability of the chromatographic method. The stock solutions of 9.2×10^{-4} M PER, 8.8×10^{-4} M AML, and 1.37×10^{-3} M IND were used in these studies. Based on the results, there were no strong signs of degradation of all compounds observed in acidic conditions. Contrary to acidic conditions, in basic media not only was PER extremely degraded by about 83.75%, but AML and IND were also affected by this condition. The situation was quite different in the case of oxidative treatment: nearly no degradation products occurred for PER and AML. In the case of IND, only 3.3%

degradation was determined. Not only the heating of the solid form but also the solution in water bath affected production of degradation peaks. In both conditions, degradation appeared within the range of 4.55%–12.7% and 5.83%–14.59% for heating the solid form in an oven and heating the solution in a water bath, respectively.

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