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Zero crossing and ratio spectra derivative spectrophotometry for the dissolution tests of amlodipine and perindopril in their fixed dose formulations

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ABSTRACT

Dissolution tests of amlodipine and perindopril from their fixed dose formulations were performed in 900 mL of phosphate buffer of pH 5.5 at 37°C using the paddle apparatus. Then, two simple and rapid derivative spectrophotometric methods were used for the quantitative measurements of amlodipine and perindopril. The first method was zero crossing first derivative spectrophotometry in which measuring of amplitudes at 253 nm for amlodipine and 229 nm for perindopril were used. The second method was ratio derivative spectrophotometry in which spectra of amlodipine over the linearity range were divided by one selected standard spectrum of perindopril and then amplitudes at 242 nm were measured. Similarly, spectra of perindopril were divided by one selected standard spectrum of amlodipine and then amplitudes at 298 nm were measured.
Both of the methods were validated to meet official requirements and were demonstrated to be selective, precise and accurate. Since there is no official monograph for these drugs in binary formulations, the dissolution tests and quantification procedure presented here can be used as a quality control test for amlodipine and perindopril in respective dosage forms.

INTRODUCTION

Most of the hypertensive population requires the treatment with two or more antihypertensive agents. Rational combinations in this area are based on agents that either interfere with different pathophysiological mechanisms or effectively block respective responses [8]. An example of synergistic action in this area is the use of the calcium channel blocker, e.g. amlodipine and the angiotensin convertase enzyme inhibitor, e.g. perindopril (Fig. 1).

As far as previous analytical procedures are concerned, some HPLC methods are now available for simultaneous determination of amlodipine and perindopril [1,3,6,7,12]. As concerns spectrophotometry, only one method for simultaneous determination of both of the drugs had been described previously [5]. From among the methods mentioned above, only one HPLC method is proposed for the dissolution study of amlodipine and perindopril in their fixed dose formulations [3]. However, to the best of our knowledge no spectrophotometric procedure has been described as a suitable tool for this purpose.

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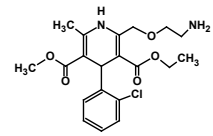
Amlodipine



Perindopril

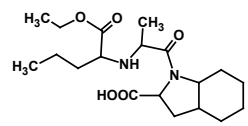


Figure 1. The chemical structures of amlodipine and perindopril

Meanwhile, spectrophotometric methods are still recommended for the dissolution studies because of availability of the instrumentation, the simplicity, speed, precision and accuracy of these techniques [9].

Therefore, the present study was undertaken to prepare new reliable spectrophotometric procedures, which could be useful for quantification of amlodipine and perindopril in their fixed dose formulations, including the dissolution tests.

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These drugs have similar zero order spectra in the UV region (Fig.2), so two derivative spectrophotometric techniques suitable for extracting information from the overlapping bands were proposed [4,11].

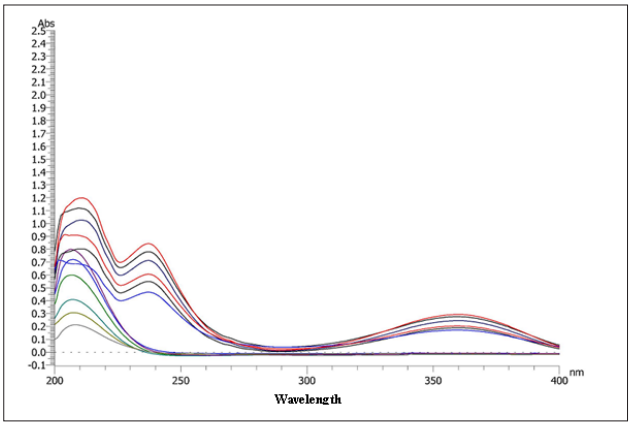

Figure 2. The zero order overlain spectra of amlodipine and perindopril (2-12 µg/mL) in the UV region

MATERIALS AND METHODS

Materials and reagents

Amlodipine besylate and perindopril erbumine (tert-butyl amine) pure substances were purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA). Also, Amlessa® tablets containing 10 mg of amlodipine and 8 mg of perindopril from Krka (Krakow, Poland) were used. The declared excipients for this formulation were: sodium bicarbonate, microcrystalline cellulose, gelatinized mays starch, sodium carboxy methyl starch A, colloidal silica anhydrous and magnesium stearate. Methanol of spectroscopic grade purity was purchased from E. Merck (Germany). All other chemicals were of analytical grade and were supplied by Sigma Chemicals Co. (St. Louis, MO).

Stock solutions of amlodipine and perindopril were prepared by dissolving 10 mg of amlodipine and perindopril in methanol to obtain the concentration of 1 mg/mL. Then, respective dilutions were done using phosphate buffer at pH 5.5. This buffer was prepared according to the European Pharmacopoeia 7th Edition.

Equipment

Evolution 6100 dissolution system from Distek Inc. (North Brunswick, NJ, USA) was used for the dissolution studies. The pH measurements were performed with a model HI9024C of pH-meter from Hanna Instruments (Villafranca Padovana, Italy). Hitachi UV/Vis U-2001 (Tokyo, Japan) double beam spectrophotometer connected to a computer loaded with Hitachi software was used for all spectrophotometric measurements. They were carried out in 1 cm quartz cells using phosphate buffer of pH 5.5 as blanks.

Stability of amlodipine and perindopril in phosphate buffer of 5.5

Samples of amlodipine and perindopril in phosphate buffer of pH 5.5 were heated in a water bath at 37°C under continuous stirring. Respective volumes were taken at the time intervals of 30, 60 and 90 min, diluted to gain the concentration over the linearity range and measured in the UV range of 200 to 400 nm.

Calibration

The stock solutions of amlodipine and perindopril were diluted with phosphate buffer of pH 5.5 to obtain the working concentrations over the range 2-12 µg/mL. The absorption spectra of these amlodipine and perindopril mixtures were recorded in the range of 200 nm to 400 nm using a bandwidth of 2 nm and a scan speed of 400 nm/min. Spectra were stored in the memory of the instrument and transformed to their first derivative with $\Delta\lambda$=4 nm and scaling factor equal 20.

Zero crossing derivative spectrophotometry

First derivative spectra of amlodipine and perindopril showed that perindopril had zero crossing point at 253 nm and at this wavelength amlodipine was determined. In turn, 229 nm was the wavelength suitable for the determination of perindopril (Fig.3). For the preparation of calibration graphs the amplitudes obtained at 253 nm were plotted against the respective concentrations of amlodipine (2-12 µg/mL) and the amplitudes at 229 nm were plotted against the respective concentrations of perindopril (2-12 µg/mL).

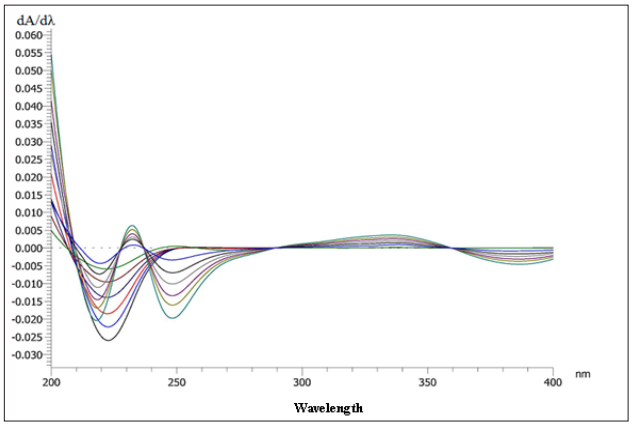

Figure 3. The first derivative overlain spectra of amlodipine and perindopril (2-12 µg/mL) with amplitudes at 253 nm for amlodipine and 229 nm for perindopril

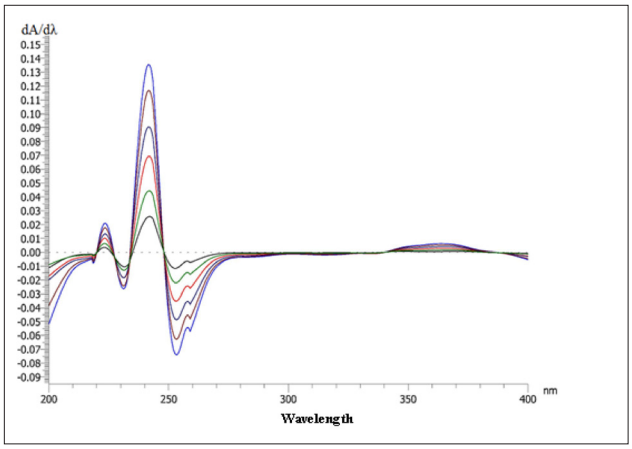

Figure 4. The first derivative spectra of amlodipine over the range 2-12 µg/mL divided by one selected standard spectrum of perindopril (12 µg/mL) with amplitudes at 242 nm

Ratio spectra derivative spectrophotometry

First derivative spectra of amlodipine over the range 2-12 µg/mL were divided by one selected standard spectrum of perindopril (2 µg/mL) and then amplitudes at 242 nm were measured (Fig.4.). Similarly, first derivative spectra

of perindopril over the range 2-12 µg/mL were divided by one selected standard spectrum of amlodipine (2 µg/mL) and then amplitudes at 298 nm were measured (Fig. 5).

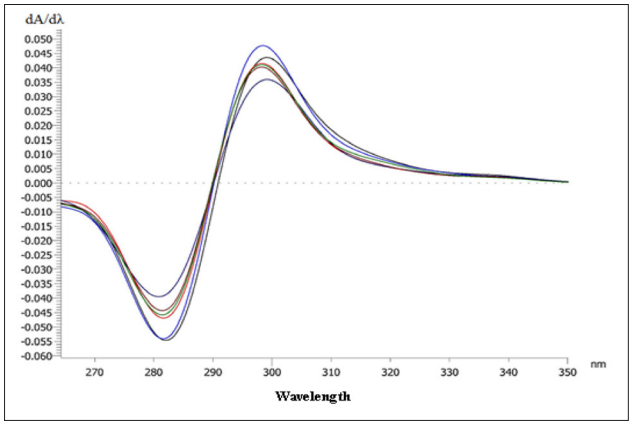

Figure 5. The first derivative spectra of perindopril over the range 2-12 µg/mL divided by one selected standard spectrum of amlodipine (2 µg/mL) with amplitudes at 298 nm

Limit of detection (LOD) and limit of quantification (LOQ)

For both of the techniques, LOD and LOQ were calculated using the formulas based on SD of the intercept of the respective regression line [10].

Precision

Precision of the methods was evaluated by analyzing the mixtures of amlodipine and perindopril at three different concentrations 3, 7 and 11 µg/mL for both the drugs. These solutions were analyzed three times in the same day (for intra day precision) and over a period of three days (for inter day precision). Finally, the precision was expressed by respective RSD values.

Accuracy in the fortified samples

The amounts of Amlessa® tablets of ca. 0.1 g were transferred to 50 mL volumetric flasks with ca. 30 mL of methanol, sonicated for 30 min, diluted to the mark with methanol, mixed and filtered by nylon membrane filters (0.45 µm). Volumes of 0.8, 1.0 and 1.2 mL of the filtrates were used to fortify the standard mixtures of amlodipine and perindopril at 80, 100 and 120% levels of fortification. These fortified samples were diluted with phosphate buffer of pH 5.5 to gain the linearity range and analyzed by the described methods.

Assay in tablets

The amounts of Amlessa® tablets of ca. 0.1 g were transferred to 50 mL volumetric flasks with ca. 30 mL of methanol, sonicated for 30 min, diluted to the mark with methanol, mixed and filtered by nylon membrane filters (0.45 µm). Then, 1.5 mL of the filtered solutions were transferred to 10 mL volumetric flasks, diluted with phosphate buffer of pH 5.5 and analyzed by the described methods.

Dissolution studies

The dissolution test was performed using 900 mL of phosphate buffer of pH 5.5 at 37°C, in the paddle apparatus with a frequency of rotation equal of 100 rpm. The dissolution medium was degassed by heating, filtering and drawing a vacuum for a short period of time. For each 900 mL of the medium one tablet containing 10 mg of amlodipine and 8 mg of perindopril were used. After 45 min of the test, volumes of ca. 10 mL of each sample were taken, filtered by nylon membrane filters (0.45 µm) and analyzed by the proposed spectrophotometric methods.

RESULTS AND DISCUSSION

Some chromatographic methods have been reported to date for the simultaneous determination of amlodipine and perindopril in their binary mixtures [1,3,6,7,12]. Also one spectrophotometric method has been developed for the same purpose [5]. However, no spectrophotometric procedure has been proposed for the dissolution study of these valuable drugs in their fixed dose formulations. In the present investigation, two different spectrophotometric techniques were elaborated for this purpose. These drugs have similar zero order spectra in the UV region so two derivative spectrophotometric methods suitable for extracting information from the overlapping bands were proposed, i.e. zero crossing and ratio spectra derivative spectrophotometry [4,11].

The instrumental parameters, i.e. smoothing factor Δλ in the range 2-5 and scaling factor in the range 10-50 were examined during the developing study. Finally, the smoothing factor equal 4 and scaling factor equal 20 were found to be optimum for tracing the first derivatives of the spectra as far as linearity of the methods is concerned. The scan speed was optimized in the range 200-1200 nm/min and then the speed equal 400 nm/min in connection with a slit width equal 2 nm was used through the work.

Amlodipine spectrum in zero order is overlapped with that of perindopril but first derivative of the spectra could resolve the overlapped peaks using zero crossing technique and measuring of amplitudes at 253 nm for amlodipine and 229 nm for perindopril giving satisfactory results for the linearity study (Tab.1).

Also, the ratio spectra derivative spectrophotometric method has been found to be useful in which the amplitudes at 242 and 298 nm were measured for amlodipine and perindopril, respectively. In this technique, an accurate choice of a divisor standard concentration is fundamental for several reasons, hence we tested the method with various divisor concentrations. It was observed that the standard solution of 2 µg/mL of perindopril was suitable for determination of amlodipine and 12 µg/mL of amlodipine was suitable for determination of perindopril. These divisor concentrations gave the best results in terms of signal to noise ratio and the highest correlation coefficient values for the calibration procedure (Tab.1).

Table 1. The linearity study for amlodipine and perindopril for the zero crossing and ratio spectra derivative methods (n=6)

Equationy=ax + b		LOD (µg/mL)	LOQ (µg/mL)	r	Mandel testF	p
Zero crossing						
Amlodipine	y=0.00157x + 0.0008	0.32	0.96	0.9973	1.2451	0.1369
Perindopril	y=0.00174x + 0.0017	0.29	0.89	0.9961	1.0236	0.1526
Ratio spectra derivative						
Amlodipine	y=0.01129x + 0.0016	0.27	0.81	0.9985	1.2265	0.1357
Perindopril	y=0.01720x + 0.0361	0.33	0.99	0.9960	1.0059	0.1436

In zero crossing method the inter day precision expressed as RSD was from 1.58% to 0.19% for the lowest and the highest concentration of amlodipine. The respective values for perindopril were in the range 1.17-0.28%. In ratio spectra derivative method the inter day RSD values ranged from 1.14% to 0.18% for amlodipine, and from 1.50% to 0.28% for perindopril. Taking together, RSD values obtained for both the drugs and both the methods confirmed that the applied procedures were sufficiently precise (Tab.2).

Table 2. The precision study for amlodipine and perindopril for the zero crossing and ratio spectra derivative methods

Declared(μg/mL)		Intra day precision (n=3)		Inter day precision (n=9)	
		Determined Mean±SD	RSD (%)	Determined Mean±SD	RSD (%)
Zero crossing					
Amlodipine	3	2.51±0.03	1.19	2.53±0.04	1.58
	7	6.62±0.02	0.30	6.65±0.03	0.45
	11	10.69±0.02	0.19	10.71±0.02	0.19
Perindopril	3	2.54±0.03	0.79	2.56±0.04	1.17
	7	6.69±0.01	0.15	6.71±0.02	0.30
	11	10.72±0.02	0.19	10.74±0.03	0.28
Ratio spectra derivative					
Amlodipine	3	2.61±0.02	0.76	2.64±0.03	1.14
	7	6.73±0.02	0.30	6.77±0.03	0.44
	11	10.84±0.03	0.18	10.86±0.02	0.18
Perindopril	3	2.63±0.02	0.76	2.67±0.04	1.50
	7	6.75±0.02	0.30	6.80±0.03	0.44
	11	10.85±0.02	0.28	10.89±0.03	0.28

The analysis of commercially available formulations using the above procedures revealed satisfactory results as evident from the results shown in Table 3. Also, the fortified samples with different concentrations of amlodipine and perindopril were analyzed. The percentage recoveries obtained for each level of fortification are given in Table 4.

Table 3. The results obtained for amlodipine and perindopril in the fortified samples

Drug	Level of fortification (%)	Declared (μg/mL)	Determined Mean±SD (n=3)	RSD (n=3)	Recovery (%) (n=9)
Zero crossing					
Amlodipine	80	7.68	7.62±0.04	0.53	99.26
	100	9.10	9.05±0.04	0.40	99.45
	120	10.52	10.48±0.03	0.29	99.59
Perindopril	80	6.56	6.45±0.05	0.78	98.37
	100	7.70	7.63±0.04	0.55	99.13
	120	8.84	8.80±0.03	0.29	99.51
Ratio spectra derivative					
Amlodipine	80	7.68	7.62±0.01	0.14	98.00
	100	9.10	9.06±0.04	0.08	99.37
	120	10.52	10.49±0.01	0.07	99.60
Perindopril	80	6.56	6.43±0.01	0.14	98.37
	100	7.70	7.65±0.01	0.09	99.13
	120	8.84	8.81±0.01	0.09	99.51

All obtained results were homogenic and the t Student test did not show significant differences between them and the declared contents of the drugs. The results were also estimated by calculating the 95% confidence intervals and checking if the determined amounts were inside them. For both the drugs and both the methods, all contents were in the confidence intervals so our determinations were sufficiently accurate.

Table 4. The results obtained for amlodipine and perindopril determination in Amlessa® tablets (n=6)

Drug	Declared (μg/ml)	Determined 95% Confidence Interval (μg/ml)	Determined Mean±SD (μg/ml)	Recovery Mean (%)	Student test *t*	*p*
Zero crossing						
Amlodipine	10.65	10.67-10.75	10.71±0.04	100.56	4.1948	0.009
Perindopril	8.55	8.44-8.52	8.48±0.03	99.18	-5.0344	0.004
Ratio spectra derivative						
Amlodipine	10.65	10.76-10.86	10.81±0.04	101.50	8.7885	0.000
Perindopril	8.55	8.38-8.47	8.43±0.04	98.60	-6.8006	0.001

All obtained results were homogenic and the t Student test did not show significant differences between them and the declared contents of the drugs. The results were also estimated by calculating the 95% confidence intervals and checking if the determined amounts were inside them. For both the drugs and both the methods, all contents were in the confidence intervals so our determinations were sufficiently accurate.

The main purpose of the present study was to apply the elaborated methods for the dissolution studies of fixed dose formulations containing amlodipine and perindopril. It is widely accepted that dissolution testing is a very important tool in the pharmaceutical industry for providing valuable information to design new products and to ensure respective drug quality. It is necessary to have such a test for controlling that the dissolution properties are consistent both within a manufactured bath and between bathes. A dissolution test is also a valuable tool in a bioequivalence study of generic products when a similarity of dissolution profiles between the potential generic product and the reference should be demonstrated [2]. According to European Pharmacopoeia 7th Edition, no less than 80% of the active ingredients of the labeled claim should be dissolved for a conventional oral formulation.

In our experiment, the choice of the dissolution medium was difficult due to differences in chemical properties of amlodipine and perindopril and their low solubility. After many initial tests, phosphate buffer of pH 5.5 was chosen as a compromise for both the drugs. The tablets were treated with 900 mL of the buffer at 100 rpm as the paddle speed and temperature of 37°C during 45 min. These experiments revealed satisfactory results for both the drugs and two elaborated methods as evident from the results shown in Table 5. After dissolution, the percentage recoveries of both the drugs were far above minimal values indicated in official requirements, e.g. European Pharmacopoeia 7th Edition (80% of the label claimed). The results were also estimated by calculating the 95% confidence intervals and checking if the determined amounts were inside them. For both the drugs and both the methods, all contents were in the confidence intervals so our determinations of the dissolved drugs were sufficiently accurate.

Table 5. The results obtained for amlodipine and perindopril during the dissolution study from Amlessa® tablets (n=6)

Drug	Declared (μg/ml)	Determined 95% Confidence Interval (μg/ml)	Determined Mean±SD (μg/ml)	Recovery Mean (%)	Student test *t*	*p*
Zero crossing						
Amlodipine	11.11	10.88-11.18	11.11±0.14	99.28	3.8531	0.012
Perindopril	8.88	8.58-9.02	8.80±0.21	99.15	-0.8739	0.02
Ratio spectra derivative						
Amlodipine	11.11	10.95-11.03	10.99±0.04	98.98	1.1299	0.041
Perindopril	8.88	8.39-9.21	8.80±0.04	99.10	-5.2588	0.003

For each component, the results obtained by the proposed methods were statistically compared using the single factor analysis of variance test (one way ANOVA). The calculated F value did not exceed the critical value for any of the two drugs, indicating that there is no significant difference between the proposed methods (data not shown).

In conclusion, rapid, precise, accurate and specific spectrophotometric methods for the simultaneous determination of amlodipine and perindopril in combined pharmaceuticals were elaborated. The minimum sample preparation, speed of analysis and low cost are the main advantages of these methods when compared to other methods such as HPLC.

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