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RESEARCH ARTICLE

Simultaneous Determination of Lisinopril and Amlodipine Using Third Derivative Spectroscopy

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ABSTRACT

In the present investigation, the simultaneous determination of lisinopril and amlodipine in their pure forms and in pharmaceutical formulations were determined using third derivative spectrophotometry. The process is easy, precise, accurate, and economical. The medications in the combined formulation were analyzed using the zero-crossing point method. Lisinopril and amlodipine were discovered to have wavelengths of (204 and 240 nm) and (248 and 272 nm), respectively, in solvent medium in a concentration range of 5–45 $\mu\text{g mL}^{-1}$ for lisinopril and 5–40 $\mu\text{g mL}^{-1}$ for amlodipine. The results were much in line with the accepted methodology. Common tablet excipients showed no interference. The recovery rate of lisinopril dihydrate was 98.57 to 103.42% and amlodipine 95.14 to 102.80%. Amlodipine besylate and lisinopril dihydrate were satisfactorily measured using this technique in tablet forms.

Keywords: Amlodipine, Combined formulation, Lisinopril, Third derivative spectroscopy, Zero-crossing point**Introduction**

When two chemicals' zero-order spectra are similar, it may be challenging to resolve them in this way. Derivative spectroscopy can be used to solve the latter issue. Compared to zero-order spectra, derivatives of spectra offer better resolution but a lower signal-to-noise.¹ The derivative technique is frequently used to resolve binary mixtures of components with overlapped spectra on the basis of zero-crossing measurements,² or compensation technique.³

Amlodipine besylate (AMD) "2-[(2-Aminothoxy) methyl]-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5-pyridin diacarbonylic acid 3-ethyl 5-methyl ester benzene sulfonate" is an effective antihypertensive medication belonging to dihydropyridine calcium channel blocker. This group of antihypertensives have a less incidence of myocardial failure and impaired cardiac conduction than other calcium channel blockers because of their selectivity for the peripheral blood vessels. AMD is frequently used to treat angina and excessive blood pressure.

Amlodipine's possibility for a single daily dose is one of its appealing qualities.³

Lisinopril Dihydrate (LSD) "(2S)-1-[(2S)-6-amino-2-[[[(1S)-1-carboxy-phenyl propyl] amino} hexanoyl] pyrrolidin-2-carboxylic acid dihydrate" a synthetic peptide derivative and an antihypertensive angiotensin-converting enzyme (ACE) inhibitor that works by directly inhibiting angiotensin-II production while also boosting bradykinin levels. Furthermore, angiotensin II-stimulated aldosterone production by the adrenal cortex is reduced, resulting in lower salt and water retention as well as a rise in blood potassium.^{4,5}

When used together, the two long-acting medication classes of amlodipine and lisinopril significantly lower blood pressure and have fewer adverse effects than when taken separately and this has been achieved after developing the lisinopril/amlodipine tablets (Lisonorm[®], 10 mg/5 mg), which are currently approved in several European Union nations.^{6,7} Even though a large number of analytical studies for amlodipine and lisinopril as a single

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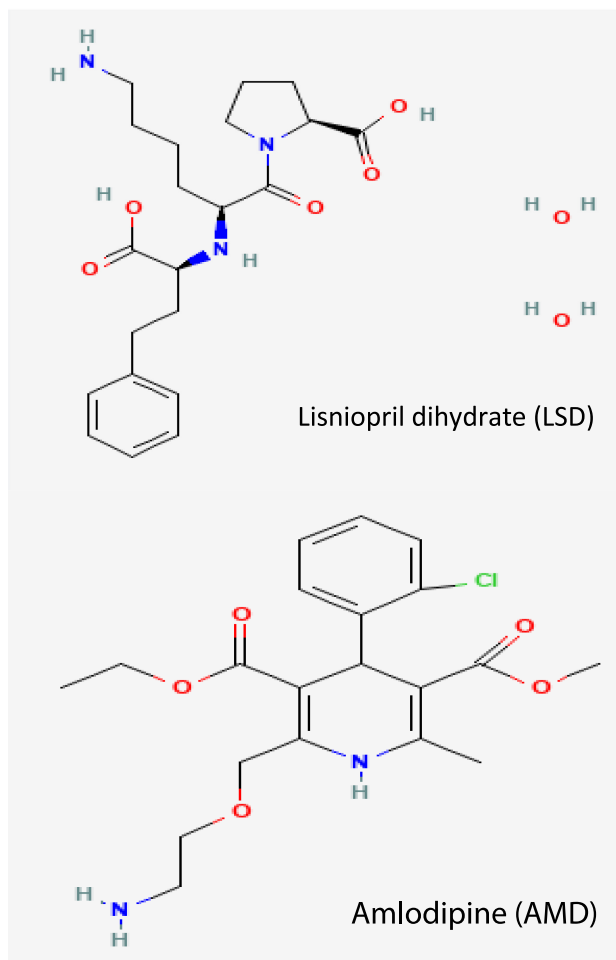


Fig. 1. Chemical structures of lisinopril dihydrate (LSD) and amlodipine (AMD). (Adapted from Pubchem).⁷

tablet have been published, very few of them were combination-focused, Fig. 1.⁷

Potentiometry,⁸ Non-aqueous titration,⁹ and HPLC,^{10,11} are the approved methods for estimating AMD and LSD, respectively. HPLC,^{12,13} HPTLC,^{14,15} UV-Spectrophotometric^{15–17} and Spectrofluorimetric techniques have been published for simultaneous quantification of either drug in combination with other pharmaceuticals.^{18,19} LSD and AMD were also determined using the first or second derivative spectrum technique and provided precise results free of interference.^{20,21} Recently Nejres and Najem reported a 4th derivative spectroscopy method for the simultaneous determination of these two APIs.²²

The present method uses distilled water as a solvent to estimate both drugs simultaneously utilizing a simultaneous equation and UV-Spectrophotometry. The new approach employs simple and low-cost reagents and methods to achieve sensitivity comparable to that of challenging and costly techniques such as HPLC and HPTLC.

Materials and methods

Instruments

All spectrum and absorbance measurements were performed using a double beam Labomed Inc 2602-UV/Vis spectrophotometer with wavelength precision of 2 nm, and matched quartz cells with a 1 cm optical path length. pH measurements were performed using a HANNA brand pH211 model pH meter.

Materials

All compounds employed were of analytical reagent grade, and the solvent medium was prepared using double distilled water. Pharmaceutical grade AMD (99% purity, CAS 88150-42-9) were provided by Energy Chemical Company (China) and LSD (99% purity, CAS 83915-83-7) provided by Meryer Company (China) and used as supplied. These stock solutions (100.0 mg/ml) were diluted with double distilled water to achieve working concentrations ranging from 10.0 to 100.0 $\mu\text{g ml}^{-1}$ for AMD and 20.0 to 100.0 $\mu\text{g ml}^{-1}$ for LSD. Hipril A tablets (Micro Labs Ltd, India) was purchased from local pharmacy in Mosul, Iraq.

UV derivative spectroscopic method

Standard AMD and LSD solutions were scanned using a Labomed UV-visible spectrophotometer in the 200–300 nm wavelength range with a slow scan speed in the absorbance measurement mode and a preset interval of 0.2 nm. An example was provided using distilled water. The UVWin7 program (V5.2.0.1104) was employed to transform the zero-order spectrum to third derivative using 21 points of derivative once the spectrophotometer was connected to the computer.

Preparation of standard solutions

A 10.88 mg LSD standard, or 10 mg of lisinopril, was precisely weighed and then transferred to a beaker. There, it was dissolved in 30 mL of distilled water and then added to a volumetric flask to make 100 mL (0.1 mg ml^{-1}). A dose of 13.86 mg AMD, or 10 mg of amlodipine, was weighed accurately and put into a beaker. It was then dissolved in 30 mL of distilled water and diluted to a total of 100 mL in a sealed volumetric flask (0.1 mg mL^{-1}). The medicine solutions were kept in the refrigerator at 8 °C.

Assay of pharmaceutical formulation

Ten tablets (Hipril A) containing five milligrams of amlodipine and lisinopril were weighed and ground

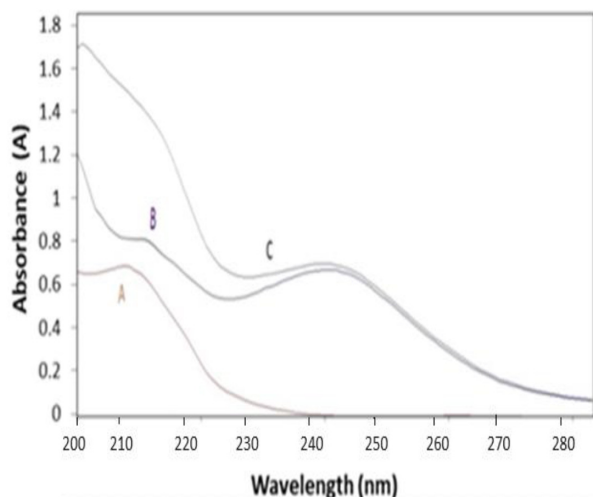


Fig. 2. Zero-order spectra of lisinopril (A), amlodipine (B), and amlodipine with lisinopril in 0.1 M HCl (C).

into a fine powder. A powder of an equivalent weight of one tablet was transferred into a 25 mL beaker, followed by adding 10 mL of distilled water and 1 mL of 1 M HCl. The mixture was sonicated for 20 minutes with shaking. The precipitate on filter paper was washed with distilled water several times before being added to the filtrate. The filtrate volume was increased to 100 mL in a volumetric flask using distilled water.

Results and discussion

At 205 nm, LSD appears to have a single maximum absorbance band. AMD, on the other hand, exhibits two maximum absorbance bands with a shoulder at 212 and 238 nm, but the spectrum of the two pharmaceutical substances combined generated a band at 235 nm with a shoulder at 216 nm as seen in Fig. 2. The simultaneous determination of LSD-AMD binary combinations in their pharmaceutical dosage forms was not possible with the zero-order spectrum because of insufficient sensitivity and interference from the dosage form excipients and each other.

The derivative spectrophotometry technique can be used to determine different compounds whose spectra overlap with the least amount of error. Both drug's zero-order spectra were found to overlap, and each substance impedes the spectrophotometric analysis of the other. However, this spectral overlapping was sufficient to demonstrate the proposed method's resolving power. The derivative method was chosen and used due to its spectral properties, selectivity, and sensitivity. The derivative spectra enabled the identification of two drugs at the same time. But the primary benefit of derivative spectroscopy might

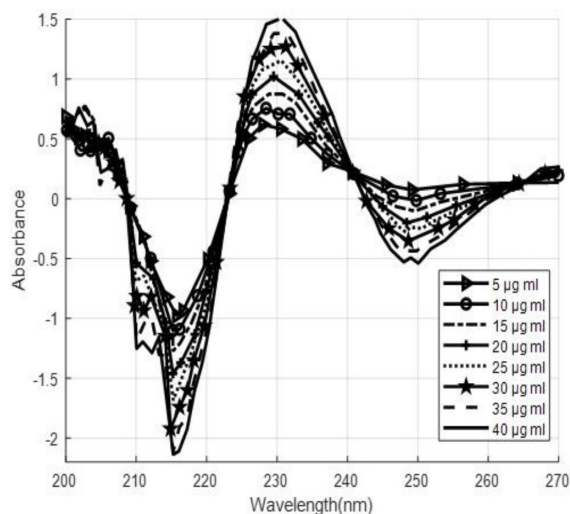


Fig. 3. Third derivative spectroscopy of various concentrations of amlodipine ($5 \mu\text{g ml}^{-1}$ – $40 \mu\text{g ml}^{-1}$) Simultaneous Determination of Lisinopril and Amlodipine.

be the opportunity to do measurements in peak correspondence; as a result, it may be more sensitive and accurate.

The zero-crossing point approach is used to achieve this. It is described as a certain wavelength at which one component responds (positively or negatively), but not other substances. At a zero-crossing point, one component's reaction can be quantified while the other's response is zero. Nevertheless, for simultaneous estimation of both medications, there must be two zero crossing sites where one component's response can be quantified while the other's response is zero.^{23,24}

Third derivative spectroscopy of AMD

Fig. 3 depicts the third derivative spectroscopy of AMD. AMD has two peaks at 215 nm and 248 nm that are negative, and two peaks at 230 nm and 272 nm that are positive. Although the medicine has several zero-crossing points, the most practical one is at 222 nm, where the drug exhibits zero absorbance at various doses.

Third derivative spectroscopy of lisinopril

The third derivative spectroscopy of LSD concentrations is shown in Fig. 4. Positive peaks for LSD are located at 206 nm and 228 nm, whereas the negative peak is located at 215 nm. When the drug is present alone, these peaks could be utilized to identify it. The medication has multiple zero crossing points, but the most significant one is at 223 nm, where the dA^3/d^3 is zero. The zero-crossing wavelengths must remain

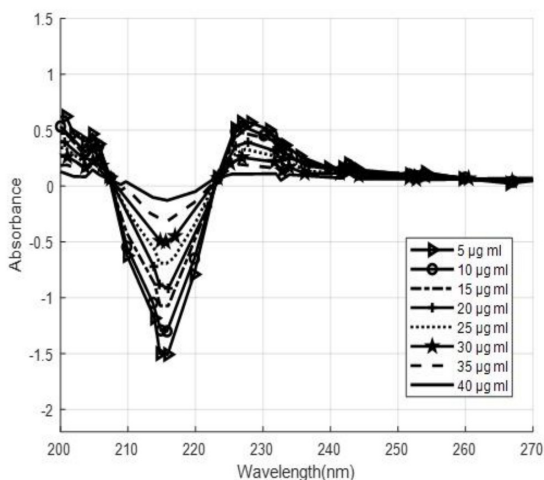


Fig. 4. Third derivative spectroscopy of different concentration of lisinopril ($5 \mu\text{g ml}^{-1}$ – $45 \mu\text{g ml}^{-1}$) Calibration Graphs for Analysis of LSD and AMD.

Table 1. Comparison of effective working range displayed by different analytical methods.

Analytical method	Working range ($\mu\text{g/mL}$)	Working range ($\mu\text{g/mL}$)	Reference
	Amlodipine	Lisinopril	
RP-HPLC	10–50	10–50	10
RP-HPLC	12.5–40	25–80	11
Derivative spectroscopy	2.5–12.5	5–15	20
4 th derivative spectroscopy	2–35	2–45	22

constant while the test analyte concentration changes to use the zero-crossing derivative approach. To evaluate the condition, variations in the zero-crossing wavelengths for AMD were investigated.

Table 1 shows the working range for determination of LSD-AMD in previous analytical methods. AMD could be identified in combined dose since it was detected at 240 nm in the presence of LSD at a concentration of $5 \mu\text{g/ml}$, which has no signals. Similarly, LSD has been detected at 223 nm in the presence of $30 \mu\text{g.ml}^{-1}$ AMD. Because Fig. 4's depiction of the latter zero-crossing point shows, LSD can be calculated even at higher concentrations of AMD.

The calibration graphs were made between third derivative response and concentrations for various runs. All calibration graph parameters including linearity, concentration range and regression coefficient are tabulated in Fig. 5. Calibration curves for both LSD and AMD at different wavelengths demonstrated linearity values above 0.99.

Precision and accuracy

The pure drug solution was evaluated three times at two different concentrations (7 and $15 \mu\text{g mL}^{-1}$) to

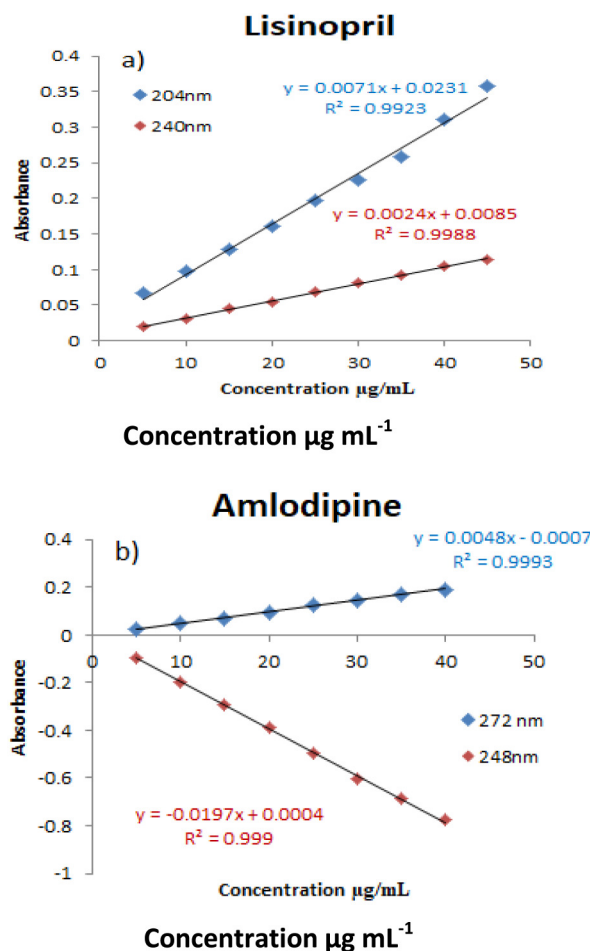


Fig. 5. (a) Calibration curve of LSD at 204 and 240 nm, (b) Calibration curve of AMD at 248, and 272 nm.

evaluate the suggested method accuracy. The relative standard deviation (RSD) was in the range of 0.41–3.5% to determine AMD in the presence of LSD. The recovery value was used to calculate the method's accuracy, which ranged from 98.57% to 103.42%. With the help of the developed derivative spectroscopy technique, binary combinations LSD-AMD can be quantified accurately and precisely in tablet dosage forms as well as in laboratory-prepared mixtures.

Limit of detection (LOD) and quantification (LOQ)

The absorbance of 10 samples of a mixed solution containing LSD and ALP drugs ($5 \mu\text{g mL}^{-1}$ each) was measured in accordance with ICH recommendations.²⁵ LOD and LOQ were computed in accordance with ICH recommendations. The method's acceptable and sensitive values are shown in Table 2.

Interference study

The 3rd derivative spectrophotometry method was used to determine the concentrations of AMD and

Table 2. The limit of detection LOD and quantification LOQ.

λ (nm)	SD	LOD ($\mu\text{g mL}^{-1}$)	LOQ ($\mu\text{g mL}^{-1}$)
204	0.029	0.1745	0.5816
240	0.036	0.2344	0.7813
248	0.018	0.3070	1.0233
272	0.008	0.3225	1.075

Table 3. Results of interference study for various possible coexisting components. The relative error percentage $\pm 5.0\%$.

Excipients	Concentration ($\mu\text{g mL}^{-1}$)	λ (nm)/The percentage of error			
		204	240	248	272
Glucose	50	0.44	2.26	0.83	0
	100	1.69	2.68	1.72	2.59
Fructose	50	0	1.42	1.77	0
	100	1.85	1.58	1.71	2.54
Sucrose	50	0.95	1.56	1.85	2.4
	100	0.89	3.91	2.74	2.56
KCl	50	0.94	0.95	2.54	0.65
	100	0.86	1.06	1.45	0.77

Table 4. Results of the assay of amlodipine \pm lisinopril in a commercial formulation by 3rd derivative spectroscopy.

		Hipril-A 5 mg/5 mg					
Series nm	LSD	AMD	Found	RSD %	E %	REC %	t-test
204 LSD	7	7	7.18	2.77	2.51	102.4	1.64
	15	15	14.27	3.57	4.85	95.2	2.5
240 LSD	7	7	6.96	2.06	0.44	99.6	0.37
	15	15	14.96	1.35	0.14	99.9	0.26
248 AMD	7	7	7.09	0.89	1.43	101.4	2.6
	15	15	15.42	3.25	2.67	102.8	1.4
272 AMD	7	7	7.06	0.55	0.84	99.9	1.7
	15	15	14.93	1.78	0.48	100.5	1.3

LSD in commercial medicines at $10 \mu\text{g mL}^{-1}$ and $10 \mu\text{g mL}^{-1}$, respectively. The procedure withstood concentrations of up to $200 \mu\text{g mL}^{-1}$ of KCl, sucrose, Arabian gum, and glucose. The relative error rate was 4.0% or less, Table 3. These results could implicate high selectivity of the developed assay for the determination of LSD-AMD combination without significant potential interference.

Method application

Using the developed method to assess the validity of the developed work, AMD and LSD levels in some commercial drugs were measured. Table 4 shows the results of the active ingredient determination in commercial drugs where Hipril A was investigated as the one of most used antihypertensive in Iraq. In the combined dose drugs, AMD was measured at 215 nm and LSD was measured at 207 nm, as in the first three samples.

The wavelengths used for AMD and LSD estimation were 215 nm and 254 nm for AMD and 207 nm and 231 nm for LSD estimation, and the average contents are shown in Table 3. Recovery rates obtained were in the range of 95.13–102.8% with RSD% in the range of 0.56–3.57% which was within the accepted limits for pharmaceutical quality control. Low RSD values and high % recovery statistics demonstrate that none of the suggested approaches are impacted by the excipients included in the formulations.

Conclusion

It was found that the developed method for determining AMD and LSD was easy, quick, precise, and accurate. The overlapping spectra were successfully resolved by the derivative technique, which allowed for the calculation of the combined dosage of AMD and LSD. It has not been necessary to perform complex steps like extraction or further cleansing with the suggested method. For the intended purpose, the suggested method underwent sufficient validation and testing.

The suggested method is simple since it calculates each constituent independently of the others and does not require solvent extraction. The process is also rapid, affordable, and environmentally safe. It could therefore be utilized in quality control laboratories where efficiency and affordability are key considerations.

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Author's declaration

- Conflicts of Interest: None.
- I hereby confirm that all the Figures and Tables in the manuscript are mine. Furthermore, any Figures and images, that are not mine, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- No potentially identified images or data are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of Nineveh.

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التقدير المتزامن لدوائي الأملوديبين و ليسينوبريل باستخدام طيف المشتقة الثالث

حسن سعد الديوه جي

قسم الكيمياء الصيدلانية ، كلية الصيدلة ، جامعة نينوى ، الموصل ، 41002 ، العراق.

الخلاصة

في الدراسة الحالية، تم قياس التراكيز الدوائية المتزامن لثنائي هيدرات ليسينوبريل وأملوديبين في أشكالهما النقية وفي التركيبات الصيدلانية باستخدام قياس الطيف الضوئي المشتق الثالث. العملية سهلة ودقيقة ودقيقة واقتصادية. تم تحليل الأدوية في التركيبة المركبة باستخدام طريقة نقطة عبور الصفر. تم اكتشاف أن ليسينوبريل وأملوديبين لهما أطوال موجية (204 و 240 نانومتر) و (248 و 272 نانومتر) ، على التوالي ، في وسط المذيب في نطاق تركيز 5-45 جم / مل و 5-40 جم / مل ، على التوالي . وكانت النتائج متماشية إلى حد كبير مع المنهجية المقبولة. لم تظهر سواغ الأفراس الشائعة أي تداخلات. كان معدل استرداد ليسينوبريل ثنائي الهيدرات 98.57 إلى 103.42% وأملوديبين 95.14 إلى 102.80%. تم قياس أملوديبين بيزيلات وثنائي هيدرات ليسينوبريل بشكل مرضي باستخدام هذه التقنية في الحبوب الدوائية

الكلمات المفتاحية: أملوديبين ، التحليل الطيفي المشتق ، ليسينوبريل، طيف المشتقة الثالثة، نقطة عبور الصفر.