Research article

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Second-derivative synchronous spectrofluorimetric determination of nebivolol hydrochloride and amlodipine besylate in their combined dosage form

F. Ibrahim, N. El-Enany, SH Shalan and R. A. Abo Shabana*

ABSTRACT: A rapid, simple, accurate and highly sensitive spectrofluorimetric method was developed for the simultaneous analysis of nebivolol hydrochloride (NEB) and amlodipine besylate (AML). The method was based on measuring the synchronous fluorescence intensity of the drugs at $\Delta\lambda$ = 40 nm in methanol. Various experimental parameters affecting the synchronous fluorescence of the studied drugs were carefully studied and optimized. The calibration plots were rectilinear over concentration ranges of 0.05–1.5 μg/mL and 0.5–10 μg/mL for NEB and AML with limits of detection (LOD) of 0.010 and 0.051 μg/mL and limits of quantitation (LOQ) of 0.031 and 0.156, respectively. The peak amplitudes (^{2}D) of the second derivative synchronous fluorimetry (SDSF) were estimated at 282 nm for NEB and at 393 nm for AML. Good linearity was obtained over the concentration ranges. The proposed method was successfully applied to the determination of the studied compounds in laboratory-prepared mixtures, commercial single and laboratory-prepared tablets. The results were in good agreement with those obtained using the comparison method. The mean percent recoveries were found to be 100.12 ± 0.77 and 99.91 ± 0.77 for NEB and AML, respectively. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: nebivolol hydrochloride; amlodipine besylate; second derivative synchronous fluorimetry; pharmaceutical preparations

Introduction

Nebivolol-HCl (NEB), 1-(6-fluoro-3,4-dihydro-2H-chromen-2-yl)-2-[[2-(6-fluoro-3,4- dihydro-2H-chromen-2-yl)-2-hydroxyethyl]amino]ethanol hydrochloride (1) (Fig. 1A), is the most β_1-selective adrenergic blocker (~3.5 times more selective than other β_1-adrenergic blockers) and thus might be the most cardio-selective blocker currently available in clinical practice. It has vasodilation β_1-blocker activity combined with a vasodilating effect mediated by the endothelial L-arginine nitric oxide pathway (2). Several analytical methods have been developed for assaying NEB, including spectrophotometry (4,5), spectrofluorimetry (5,6) and HPLC (7).

Amlodipine besylate (AML), 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl 5-methyl ester (1), is an optically pure *S*(–) isomer of amlodipine. AML is an effective antihypertensive agent for both systolic and diastolic hypertension, particularly in mild to moderate hypertension and angina. It avoids adverse effects such as headache, edema, flushing, palpitation, fatigue, nausea and somnolence associated with the administration of a racemic mixture of AML (2). Several analytical methods have been developed for assaying of AML either in its pure form or in pharmaceutical preparations and biological fluids. These methods include spectrophotometry (8,9), spectrofluorimetry (9,10) and HPLC (11).

To the best of our knowledge, no spectrofluorometric method has been yet reported for the simultaneous determination of NEB and AML in their pharmaceutical preparations or in biological fluids. The aim of this study was to develop an analytical method for simultaneous determination of NEB and AML in combined dosage form that is novel to the market. A formulation containing β-blockers and amlodipine is common in the Indian drug market, e.g. Atenolol with amlodipine.

A literature survey revealed that several of the analytical methods published for the determination of a combination of NEB and AML rely on the use of spectrophotometry (3,12–18), HPLC (19–22) and HPTLC (23–26).

In this study, a highly sensitive spectrofluorometric method was developed for the simultaneous determination of NEB and AML. The fluorescence spectra of NEB and AML were found to overlap, so it was difficult to determine their individual contents using conventional spectrofluorometry. This problem was resolved by using second-derivative synchronous fluorimetry (SDSF). Recently, derivative-synchronous fluorimetry has been utilized for the determination of different drugs in their dosage forms and in biological fluids (27–35). SDSF has several advantages over conventional fluorescence spectroscopy, including simple spectra, high selectivity and low interference (36). Because of its sharp, narrow spectrum, SDSF serves as a very simple, effective method for achieving data for quantitative determinations in a single run (37). The proposed method allowed the quantitation of NEB and AML with satisfactory accuracy and precision. The proposed methods were successfully

* Correspondence to: R. A. Abo Shabana, Department of Analytical Chemistry, Faculty of Pharmacy, University of Mansoura, 35516, Mansoura, Egypt. E-mail: rashaaboshabana@yahoo.com

Department of Analytical Chemistry, University of Mansoura, 35516, Mansoura, Egypt

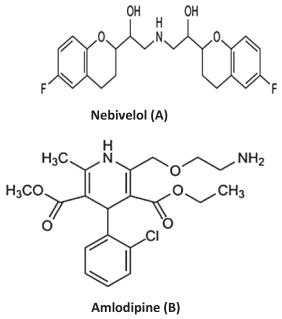

Figure 1. The structural formulae for: (A) nebivolol and (B) amlodipine.

applied to commercial single and laboratory-prepared co-formulated tablets.

Experimental

Apparatus

All fluorescence measurements were recorded with a Perkin–Elmer UK model LS 45 luminescence spectrometer, equipped with a 150 W xenon arc lamp, grating excitation and emission monochromators and a Perkin–Elmer recorder. The slit widths were 10 nm for both excitation and emission, and the photomultiplier voltage was set to auto. A quartz 1 cm cuvette was used. Derivative spectra were evaluated using fluorescence data manager software, FL WINLAB, v. 4.00.02. A Consort NV P901 digital pH meter (Turnhout, Belgium) calibrated with standard buffers was used to check the pH of the buffer solutions used.

Materials and reagents

All chemicals were of analytical reagent grade, solvents were of spectroscopic or HPLC grade and distilled water was used throughout the study.

NEB was provided by Marcyrl Pharmaceutical Industries (El Obour City, Cairo, Egypt) and was used as received without further purification. The purity of NEB was 100.02%. AML was also provided by Marcyrl Pharmaceutical Industries and was used as received without further purification. The purity of AML was 99.80%.

The following samples of NEB and AML were used:

- laboratory-prepared tablets (5 mg NEB, 5 mg AML, 20 mg talc powder, 15 mg maize starch, 15 mg lactose and 10 mg magnesium stearate per tablet);
- Nevilob® tablets – batch # 310031 (Marcyrl Pharmaceutical Industries), each labeled to contain 5 mg NEB, were obtained from commercial sources in the local pharmacy;
- Nevilob® tablets – batch # 308521 (Marcyrl Pharmaceutical Industries), each labeled to contain 2.5 mg NEB, were obtained from commercial sources in the local pharmacy; and
- Norvasc® 10 tablets – batch # 2154, labeled to contain 10 mg AML (Pfizer Egypt S.A.E., Cairo, Egypt).

Other chemicals were used as follows: methanol, acetonitrile, dimethyl sulfoxide (DMSO), dimethyl formamide (DMF) and acetone (Sigma-Aldrich, Munich, Germany); sodium dodecyl sulfate (SDS; 95%) and cetrimide (99%) (Winlab, Market Harborough, UK); hydroxy propyl β-cyclodextrin (HP-β-CD) (Merck, Darmstadt, Germany); Tween-80, methyl cellulose, acetone, ethanol, acetic acid 96%, sodium acetate trihydrate, boric acid and sodium hydroxide, 30.0% (El-Nasr Pharmaceutical Chemicals Company, ADWIC Co, Abu Zaabal, Egypt). Acetate buffer (0.2 M, pH 3.6–5.6) and borate buffer (0.2 M, pH 6.0–10.0) solutions were freshly prepared. Inactive ingredients used in the preparation of tablets (talc powder, maize starch, lactose and magnesium stearate) were also obtained from ADWIC Co.

Standard solutions

Stock solution of 100.0 μg/mL NEB and AML were prepared by dissolving 10 mg of the studied compounds in 100 mL of methanol in a calibrated flask with the aid of an ultrasonic bath. Working standard solutions were prepared by appropriate dilution of the stock solution with methanol. The solutions were stable for at least 10 days without alteration when kept in the refrigerator.

General procedures

Construction of the calibration curves. Accurately measured aliquots of NEB and AML standard solutions covering working concentration ranges of 0.05–1.5 and 0.5–10.0 μg/mL for NEB and AML, respectively, were transferred into a series of 10-mL volumetric flasks. The solutions were diluted to the mark with methanol and mixed well. Synchronous fluorescence spectra of the solutions were recorded by scanning both monochromators at a constant wavelength difference of $\Delta\lambda = 40$ nm at a scan rate of 600 nm/min using 10 nm excitation and emission windows. The second-derivative synchronous fluorescence spectra of NEB and AML were derived from the normal synchronous spectra using fluorescence data manager software (the normal synchronous was measured at 282 and 393 nm for NEB and AML, respectively). The peak amplitudes of the second-derivative spectra (^{2}D) were estimated at 282 nm for NEB and 393 nm for AML. A blank experiment was performed simultaneously. The peak amplitude of the second-derivative technique (^{2}D) of each drug was then plotted against the final drug concentration in μg/mL to give a calibration graph. Alternatively, the corresponding regression equations were derived.

Analysis of NEB/AML laboratory prepared synthetic mixtures. Aliquots of NEB and AML solutions in a ratio of 1 : 1 were transferred into a series of 10-mL volumetric flasks, diluted to the mark with methanol and mixed well. The procedure described above for construction of the calibration curves was then applied. The percentage recoveries were calculated by referring to the calibration curves or using the corresponding regression equations.

Analysis tablets. Laboratory-prepared tablets containing NEB and AML in a pharmaceutical ratio of 1 : 1 were prepared. An accurately weighed quantity of prepared tablets equivalent to 5 mg NEB and 5 mg AML (according to their pharmaceutical ratio) was transferred into a 100-mL volumetric flask and the volume was made up to the mark with methanol. For commercially available single-ingredient tablet formulations (Nevilob® and Norvasc® tablets), 10 tablets were accurately weighed and finely pulverized. A quantity of the powdered tablets equivalent to 2.5 and 5.0 mg NEB and 10.0 mg AML was transferred into a 100-mL volumetric flask and the volume was made up to the mark with methanol. The contents of the flasks were sonicated for 30 min then filtered. Different volumes of the tablet extracts were

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accurately transferred into a series of 10-mL volumetric flasks. The procedure described above for the construction of the calibration curves was then followed. The nominal content of each drug was determined either from the previously plotted calibration curve or using the corresponding regression equation.

Results and discussion

Both NEB and AML exhibit native fluorescence in methanol at 313 and 440 nm, after excitation at 284 and 355 nm, respectively, as shown in (Fig. 2). It is clear that the emission spectra of NEB and AML overlapped and analysis of such a mixture is challenging especially in their co-formulated preparation. Fig. 3 (A) shows the synchronous fluorescence spectra (SFS) of different concentrations of NEB at 282 nm in the presence of AML. Whereas Fig. 3(B) illustrates the SFS of different concentrations of AML at 393 nm in the presence of NEB. Although the synchronous fluorimetry scan resulted in sharp and narrow emission peaks for both drugs, it could not resolve the interference between their emission spectra, as shown in Fig. 3(A,B), and so it is not suitable for the simultaneous analysis of NEB and AML in a mixture. By taking the second derivative of the SFS of the studied drugs, we were able to resolve the interference successfully and we perform simultaneous estimation of NEB and AML accurately. Figs. 4 and 5 shows the SDSF spectra of NEB and AML. Using these spectra one drug can be determined at the zero crossing point of the other.

Optimization of experimental conditions

Different experimental parameters affecting the proposed method were carefully studied and optimized. The factors were changed individually, while the others were kept constant. Experimental conditions affecting the fluorescence intensity of NEB and AML were carefully considered, these included $\Delta\lambda$, pH, type of diluting solvent, type of surfactant and stability time.

Effect of pH

The influence of pH was investigated using different types of buffers covering the whole pH range, for example, 0.2 M acetate buffer (pH 3.6–5.6) and 0.2 M borate buffer (pH 7–10), in addition to 0.1 M H_2SO_4 and 0.1 M NaOH (Fig. 6).

The influence of pH on the synchronous fluorescence intensity of NEB and AML in methanol was investigated. Increasing the pH of the solution resulted in a gradual increase in the synchronous fluorescence intensity of NEB and AML up to pH 7.0, and pH 5.0 respectively, the synchronous fluorescence intensity remained constant up to pH 8.0, (and pH 6.0 for NEB and AML respectively) after which it decreased gradually. However, no

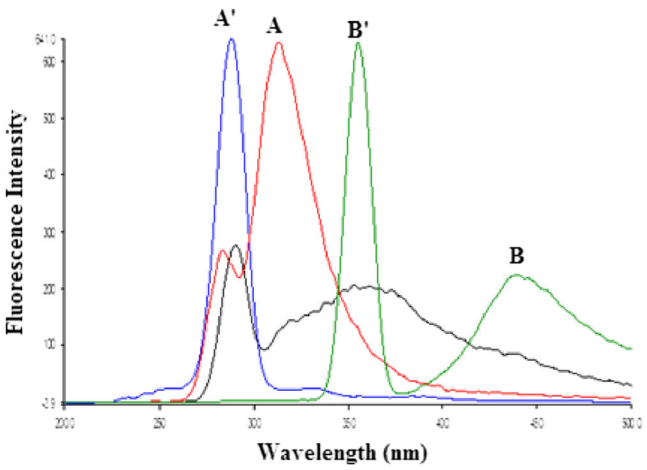

Figure 2. Excitation and emission fluorescence spectra of NEB (0.8 μg/mL) (A, A') and AML (4.0 μg/mL) (B, B') in methanol.

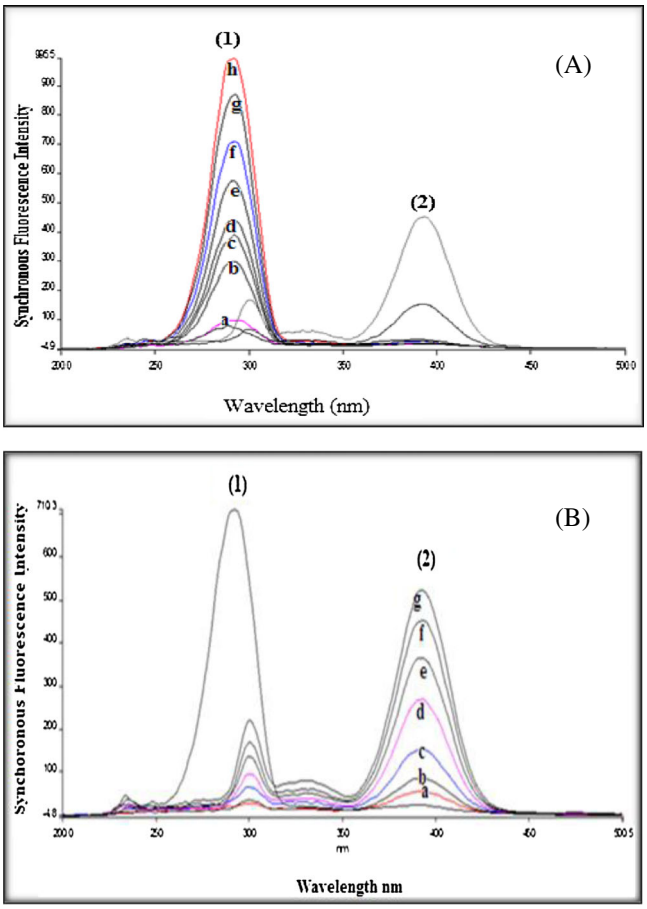

Figure 3. (A) Synchronous fluorescence spectra of (1) NEB (a–h: 0.05, 0.2, 0.5, 0.6, 0.8, 1.0, 1.2 and 1.5 μg/mL) at 282 nm and (2) AML (8.0 μg/mL. (B) Synchronous fluorescence spectra of (1) NEB (1.0 μg/mL) and (2) AML (a–h; 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 μg/mL) at 393 nm.

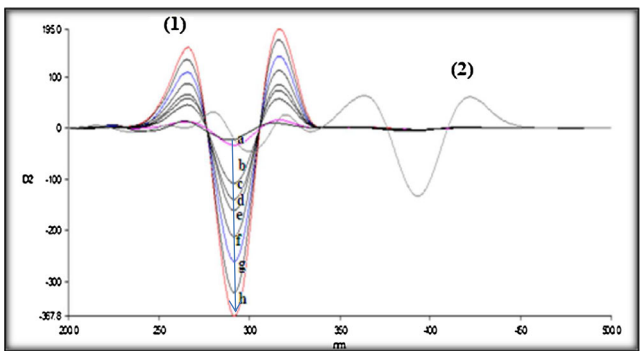

Figure 4. Second derivative synchronous fluorescence spectra of (1) NEB (a–h; 0.05, 0.2, 0.5, 0.6, 0.8, 1.0, 1.2 and 1.5 μg/mL) at 282 nm and (2) AML (8.0 μg/mL).

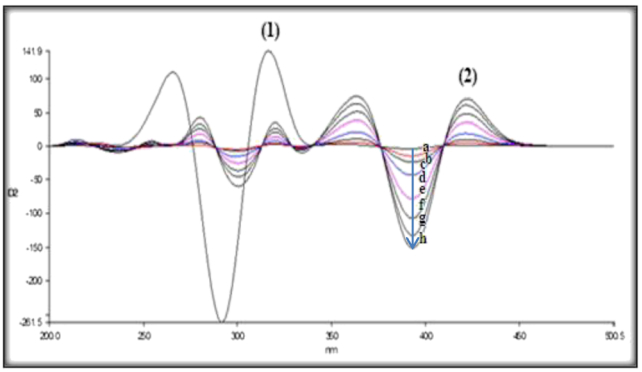

Figure 5. Second derivative synchronous fluorescence spectra of (1) NEB (1.0 μg/mL) and (2) AML (a–h; 0.5, 1.0, 2.0, 4.0, 5.0, 6.0, 8.0 and 10.0 μg/mL) at 393 nm.

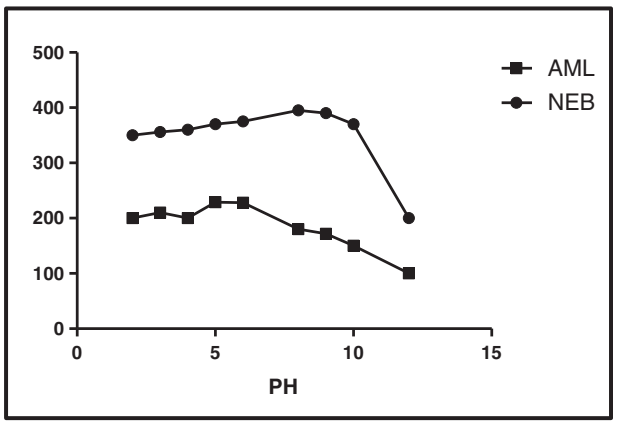

Figure 6. Effect of pH on the synchronous fluorescence intensity of NEB (0.5 μg/mL) and AML (4.0 μg/mL).

buffer was used in the study, because methanol alone resulted in a higher fluorescence intensity than buffer. Using 0.1 M H_2SO_4 resulted in high fluorescence intensity equal to that of borate buffer at pH 7.0 and lower than that achieved with methanol alone. However, using 0.1 M NaOH resulted in quenching of the fluorescence intensity.

Effect of diluting solvent

Dilution with different solvents such as water, methanol, ethanol, acetonitrile, DMSO, DMF and acetone was attempted. Both DMSO and DMF decreased the fluorescence intensities of NEB and AML, because they initiated intersystem crossing (38) (similar to a heavy atom effect). Acetone resulted in complete quenching of the fluorescence intensities of both compounds. However, the fluorescence intensities of NEB and AML were higher in water and methanol, respectively, compared with other solvents. Hence, methanol was the best solvent for dilution because it gave the highest fluorescence intensity for NEB and AML and the lowest blank reading. The results are summarized in Fig. 7.

Effect of surfactant

Different surfactants such as cetrimide (cationic surfactant), SDS (anionic surfactant), β-hydroxycyclodextrin, methyl cellulose and Tween 80 (nonionic surfactants) were tried. All these surfactants had no effect on the fluorescence intensity of NEB and AML.

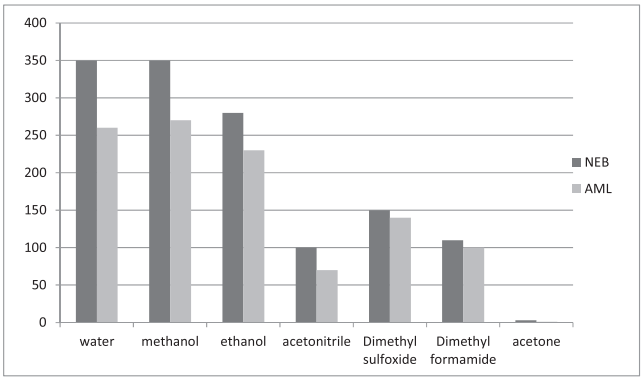

Figure 7. Effect of solvents on the synchronous fluorescence intensity of NEB (0.5 μg/mL) and AML (4.0 μg/mL).

Selection of optimum Δ λ

The SFS of NEB with AML were recorded using different values of $\Delta\lambda$. The optimum $\Delta\lambda$ value is very important for performing synchronous fluorescence scanning in terms of resolution, sensitivity and features. It can directly influence spectral shape, bandwidth and signal value. For this reason, a wide range of $\Delta\lambda$ values (20–100 nm) was examined. It was found that the optimum $\Delta\lambda$ values for NEB and AML were 45 and 40 nm, respectively. Because AML has low sensitivity relative to NEB, the optimum condition was selected to increase its sensitivity. Therefore, a $\Delta\lambda$ value of 40 nm was chosen because it resulted in the highest fluorescence intensity for AML and gave two distinct peaks of good shape for both compounds. Values of $\Delta\lambda$ lower and higher than the optimum showed low fluorescence intensity for both compounds. However, very low and very high $\Delta\lambda$ values caused irregularities in the spectral shape.

Stability of standard solutions. The effect of time on the stability of the fluorescence intensity of each compound was studied using the proposed method. It was found that the fluorescence readings remained stable for 7 days when the solutions were kept in a refrigerator at 4°C.

Validation of the method

The proposed method was tested for linearity, range, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, selectivity and sample solution stability.

Linearity. Under the above-described experimental conditions, a linear relationship was established by plotting the peak amplitude (^2D) against the drug concentration. The calibration plots were rectilinear over ranges of 0.05–1.5 and 0.5–10 μg/mL for NEB and AML at 282 and 393 nm, respectively. Linear regression analysis of the data gave the following equations:

$$^2D = 4.3232 + 104.02C (r = 0.9999) \text{ for NEB at 282 nm}$$
$$^2D = 4.5568 + 15.042C (r = 0.9999) \text{ for AML at 393 nm}$$

where ^2D is the peak amplitude in the second derivative synchronous fluorescence mode, C is the concentration of the drug in μg/mL and r is the correlation coefficient.

Statistical analysis (39) of the data gave high values for the correlation coefficients (r) of the regression equations, small values for the standard deviation of residuals ($S_{y/x}$), standard deviation of the intercept (S_a) and standard deviation of the slope

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(S_b), and small values for the percentage relative standard deviation (% RSD) and percentage relative error (% Error) (Table 1). The data proved the linearity of the calibration plots.

The results show that the proposed method is 3–24 times more sensitive than the reported chromatographic methods (19–22) for the simultaneous determination of NEB and AML. Moreover, the proposed method is 2–9 times more sensitive than the reported spectrophotometric methods (3,12–18) for NEB and AML, respectively. This high sensitivity makes the proposed method superior to the chromatographic and spectrophotometric methods reported in the literature.

Table 1. Analytical performance data for determination of the studied drugs using the proposed method

Parameter	NEB	AML
Linearity range (μg/mL)	0.05–1.5	0.5–10.0
Intercept (*a*)	4.323	4.557
Slope (*b*)	1.040×10^2	15.042
Correlation coefficient (*r*)	0.9999	0.9999
SD of residuals ($S_{y/x}$)	0.484	0.378
SD of intercept (S_a)	0.321	0.235
SD of slope (S_b)	0.371	0.042
Percent relative standard deviation (% RSD)	0.766	0.772
Percent relative error (% Error)	0.271	0.273
Limit of detection, LOD (μg/mL)	1.02×10^{-2}	5.15×10^{-2}
Limit of quantitation, LOQ (μg/mL)	3.08×10^{-2}	15.61×10^{-2}

Limit of quantitation (LOQ) and limit of detection (LOD). The LOQ was determined by establishing the lowest concentration that can be measured according to ICH Q2B recommendations (40), below which the calibration graph is nonlinear. The LOD was determined by evaluating the lowest concentration of the analytes that can be readily detected. LOQ and LOD were calculated according to ICH Q2R1 recommendations using the following equation

$$LOQ = 10\ S_a/_b \text{ and } \text{LOD} = 3.3\ S_a/_b$$

where S_a is the standard deviation of the intercept of the calibration curve and *b* is the slope of the calibration curve. The values of LOD and LOQ for NEB and AML are given in Table 1.

Accuracy. To prove the accuracy of the proposed method, the results of the assay of the studied drugs in pure form using the proposed method were compared with those obtained using the comparison method (3). Statistical analysis (39) of the results obtained by the proposed method and those obtained by the comparison method (3) using the Student's *t*-test and variance ratio *F*-test revealed no significant differences between the performance of the two methods regarding accuracy and precision (Table 2). The comparison method (3) involved a spectrophotometric method using absorbance correction, which is based on the determination of AML at 393 nm using its absorptivity value and NEB at 282 nm after deduction of the absorbance due to amlodipine besylate.

Table 2. Assay results for determination of the studied drugs in a pure form using the proposed and comparison methods

Compound	Proposed method			Comparison method (3)
	Amount taken (μg/mL)	Amount found (μg/mL)	% Found	% Found
NEB	0.05	0.051	101.40	100.81
	0.20	0.199	99.40	98.54
	0.50	0.504	100.86	100.06
	0.60	0.596	99.27	
	0.80	0.799	99.89	
	1.00	0.997	99.67	
	1.20	1.208	100.68	
	1.50	1.497	99.77	
Mean ± SD			100.12 ± 0.77	99.8 ± 1.16
t-test			0.534 (2.262)*	
F-test			2.273 (4.737)*	
AML	0.5	0.495	98.96	100.99
	1.0	0.993	99.34	98.98
	2.0	2.024	101.19	100.33
	4.0	4.018	100.46	
	5.0	4.949	98.98	
	6.0	6.013	100.21	
	8.0	8.007	100.09	
	10.0	10.001	100.01	
Mean ± SD			99.91 ± 0.77	100.07 ± 0.57
t-test			0.673 (2.262)[a]	
F-test			2.089 (4.737)[a]	

Each result is the average of three separate determinations. [a] The figures in parentheses are the tabulated *t*- and *F*-values at $P = 0.05$ (39).

Table 3. Precision data for the determination of the studied drugs by the proposed method

Parameters		NEB concentration (μg/mL)			AML concentration (μg/mL)		
		0.20	0.40	0.60	2.0	4.0	6.0
Intra-day	% Found	98.87	97.72	101.08	99.95	99.14	101.08
		100.02	100.45	100.27	101.78	99.76	99.94
		100.44	98.98	98.67	99.93	98.44	99.64
	$(\bar{x})$ ± SD	99.78 ± 0.81	99.05 ± 1.37	100.01 ± 1.23	100.55 ± 1.06	99.11 ± 0.66	100.22 ± 0.76
	% RSD	0.82	1.38	1.23	1.06	0.67	0.76
	% Error	0.47	0.80	0.71	0.61	0.39	0.44
Inter-day	% Found	101.95	100.61	99.02	101.95	98.66	99.58
		96.84	102.55	98.00	102.74	100.29	103.14
		100.85	101.27	100.19	99.94	100.77	100.82
	$(\bar{x})$ ± SD	99.88 ± 2.69	101.48 ± 0.99	99.07 ± 1.10	101.54 ± 1.44	99.91 ± 1.11	101.18 ± 1.81
	% RSD	2.69	0.97	1.11	1.42	1.11	1.79
	% Error	1.56	0.56	0.64	0.82	0.64	1.03

Each result is the average of three separate determinations.

Table 4. Assay results for determination of the studied drugs in laboratory-prepared mixtures of their pharmaceutical ratios

NEB/AML ratio	Proposed method						Comparison method (3)	
	Amount taken (μg/mL)		Amount found (μg/mL)		% Found		% Found	
	NEB	AML	NEB	AML	NEB	AML	NEB	AML
1/1 ratio	0.5	0.5	0.494	0.496	99.89	99.10	100.81	100.00
	1.0	1.0	1.011	1.009	100.05	100.90	98.54	99.50
	1.5	1.5	1.494	1.496	98.84	99.70	100.06	99.05
Mean ± SD					99.88 ± 1.15	99.90 ± 0.92	99.80 ± 1.16	99.51 ± 0.53
% RSD					1.15	0.92		
% Error					0.662	0.529		
t-test					0.082	0.643	(2.776)[a]	
F-test					1.019	3.719	(19.00)[a]	

Each result is the average of three separate determinations. [a]The figures in parentheses are the tabulated *t*- and *F*-values at $P = 0.05$ (39).

Precision

Intra-day precision. Evaluation of the intra-day precision of the proposed method was achieved through replicate analysis of three concentrations of NEB and AML in pure form on three successive times within the same day. The precision of the proposed method was proven by the small values of SD, % RSD and % Error (Table 3).

Inter-day precision. Evaluation of the inter-day precision of the developed method was performed by replicate analysis of three concentrations of NEB and AML in pure form on three successive days. Small values of SD, % RSD and % Error revealed the precision of the proposed method. The results are also summarized in Table 3.

Selectivity. The selectivity of the proposed method was established by its ability to determine the studied drugs in laboratory-prepared mixtures of NEB/AML at a ratio of 1 : 1. The second-derivative signal of NEB was measured at 282 nm, which is considered to be a zero-crossing point for AML, and that of AML was measured at 393 nm, which is the zero-crossing point for NEB (Fig. 8). From Table 4, the adequate recovered concentrations with the small values of % RSD and % error confirm the ability of the proposed method to analysis each drug in this binary mixture with acceptable analytical performance and without any interference from the anther one.

Specificity. The specificity of the proposed method was investigated by observing any interference of common tablet

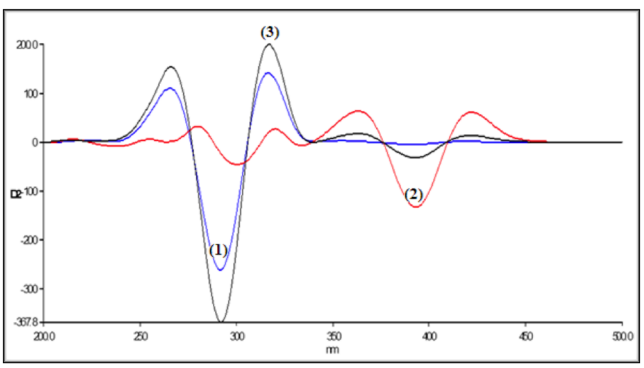

Figure 8. Second derivative synchronous fluorescence spectra of: (1) 1.0 μg/mL NEB, (2) 8.0 μg/mL AML, and (3) a mixture of 1.5 μg/mL AML and 1.5 μg/mL NEB.

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excipients such as lactose, maize starch, talc powder, magnesium stearate, calcium hydrogen phosphate and microcrystalline cellulose. These additives did not interfere with the proposed method (Table 5). The results were found to be in good agreement will the labeled amount.

Applications

Pharmaceutical application. The proposed method was successfully applied to the determination of NEB and AML in their laboratory prepared co-formulated tablets. Moreover, the

Table 5. Assay results for the determination of the studied drugs in their single tablets by the proposed method

Compound	Proposed method			Comparison method (3)
	Amount taken (μg/mL)	Amount found (μg/mL)	% Found	
Nevilob® 2.5 mg tablets	0.20	0.201	100.35	100.00
	0.60	0.598	99.63	99.36
	0.80	0.802	100.19	99.79
Mean ± SD			100.06 ± 0.38	99.72 ± 0.33
% RSD			0.378	
% Error			0.271	
t-test			1.179	(2.776)[a]
F-test			1.342	(19.00)[a]
Nevilob® 5 mg tablets	0.20	0.199	99.35	99.17
	0.60	0.604	100.65	100.22
	0.80	0.797	99.68	99.51
Mean ± SD			99.89 ± 0.68	99.63 ± 0.54
% RSD			0.677	
% Error			0.390	
t-test			0.522	(2.447)[a]
F-test			1.590	(9.277)[a]
Norvasc® 10 mg tablets	4.0	2.008	100.40	100.00
	6.0	3.984	99.60	99.21
	8.0	6.008	100.13	99.74
Mean ± SD			100.04 ± 0.41	99.65 ± 0.40
% RSD			0.407	
% Error			0.235	
t-test			1.190	(2.776)[a]
F-test			1.021	(19.00)[a]

Each result is the average of three separate determinations. [a]The figures in parentheses are the tabulated *t*- and *F*-values at $P = 0.05$ (39).

Table 6. Assay results for the determination of the studied drugs in their prepared Co-formulated tablets by the proposed method

Preparation	Proposed method						Comparison method (3)	
	Amount taken (μg/mL)		Amount found (μg/mL)		% Found		% Found	
	NEB	AML	NEB	AML	NEB	AML	NEB	AML
Prepared co-formulated tablet (1 : 1 ratio)	0.5	0.5	0.5018	0.4990	100.36	99.80	100.18	100.23
	1.0	1.0	0.9965	1.0019	99.65	100.19	99.79	98.97
	1.5	1.5	1.5018	1.4990	100.12	99.93	100.06	99.82
Mean ± SD					100.04 ± 0.36	99.97 ± 0.20	100.01 ± 0.20	99.67 ± 0.64
% RSD					0.361	0.19		
% Error					0.208	0.115		
t-test					0.139	1.179		(2.776)[a]
F-test					3.269	1.342		(19.00)[a]

Each result is the average of three separate determinations. [a]The figures in parentheses are the tabulated *t*- and *F*-values at $P = 0.05$ (39).

method was extended to the determination of the two drugs in commercial single tablets. The mean percentages found for the different concentrations were based on the average of three replicate determinations. The results shown in Tables 5 and 6 are in good agreement with those obtained using the comparison method (3). Statistical analysis of the results obtained using Student's *t*-test and the variance ratio *F*-test (39) revealed no significant difference between the performance of the proposed and comparison methods regarding accuracy and precision, respectively.

Conclusion

This study describes a fully validated and accurate second-derivative synchronous spectrofluorimetric method for the simultaneous determination of NEB and AML with enhanced sensitivity and specificity. The proposed method is less time-consuming as there is no need for elaborate derivatization reactions. This method could be applied to the analysis of NEB and AML in pharmaceutical preparations. The method can be applied to the determination of the studied drugs in quality control laboratories.

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