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Simultaneous spectrofluorimetric determination of amlodipine besylate and valsartan in their combined tablets

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Amlodipine, a dihydropyridine calcium channel blocker, and valsartan, an angiotensin II receptor blocker, are co-formulated in a single-dose combination for the treatment of hypertension. The combination is used by patients whose blood pressure is not adequately controlled on either component monotherapy. This work describes a simple, sensitive, and reliable spectrofluorimetric method for the simultaneous determination of the two antihypertensive drugs; amlodipine besylate (AML) and valsartan (VAL) in their combined tablets. The method involved measurement of the native fluorescence at 455 nm (λ_{Ex} 360 nm) and 378 nm (λ_{Ex} 245 nm) for AML and VAL, respectively. Analytical performance of the proposed spectrofluorimetric procedure was statistically validated with respect to linearity, ranges, precision, accuracy, selectivity, robustness, detection, and quantification limits. Regression analysis showed good correlation between fluorescence intensity and concentration over the concentration ranges 0.2–3.6 and 0.008–0.080 μg mL^{-1} for AML and VAL, respectively. The limits of detection were 0.025 and 0.0012 μg mL^{-1} for AML and VAL, respectively. The proposed method was successfully applied for the assay of the two drugs in their combined pharmaceutical tablets with recoveries not less than 98.85%. No interference was observed from common pharmaceutical additives. The results were favourably compared with those obtained by a reference spectrophotometric method.

Keywords: amlodipine besylate; valsartan; spectrofluorimetry; simultaneous determination; pharmaceutical tablets

Introduction

Amlodipine besylate (AML) (Figure 1), chemically known as 3-ethyl-5-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methylpyridine-3,5-dicarboxylate benzenesulphonate,[1] is a dihydropyridine calcium channel blocker used in the treatment of hypertension and angina pectoris.[1] AML is an official drug in the British Pharmacopoeia (BP) which suggests a high performance liquid chromatography (HPLC) procedure for the assay of the bulk powder.[2] Several analytical methods have been reported in the scientific literature for the determination of AML in various matrices including pharmaceutical formulations and/or biological fluids. Reported methods include various spectrophotometric methods,[3–5] anodic stripping voltammetry,[6,7] HPLC,[8,9] HPLC with fluorescence detection after chemical derivatization,[10] HPLC with amperometric detection,[11] liquid chromatography-mass spectrometry (LC-MS),[12,13] high performance thin layer liquid chromatography (HPTLC),[14] capillary electrophoresis,[15] and micellar electrokinetic chromatography.[16] Few spectrofluorimetric methods can be found in the AML literature; derivatization of the drug with several fluorogenic reagents prior to its spectrofluorimetric measurement has been reported.[17,18] Finally, the native fluorescence of AML was studied and applied for its determination in its single component dosage forms and spiked human plasma.[19]

Valsartan (VAL) (Figure 1), chemically known as N-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]-N-valeryl-L-valine[1], is an angiotensin II receptor antagonist used in the management of hypertension, to reduce cardiovascular mortality in myocardial infarction patients and in the management of heart failure.[1] The United States Pharmacopeia (USP) recommends HPLC procedures for the assay of the bulk drug as well as its mixture with hydochlorothiazide.[20] VAL was assayed in pharmaceutical formulations and/or biological samples by a variety of methods including spectrophotometry,[21–23] differential-pulse and square-wave stripping voltammetry,[24] HPLC,[25,26] HPLC with fluorescence detection,[27–29] LC-MS,[30] HPTLC,[31] capillary electrophoresis[32] and micellar electrokinetic chromatography.[33] Only one report described the native spectrofluorimetric determination of VAL in human urine samples.[34] Besides, another report made use of the native fluorescence of several angiotensin II receptor antagonists including VAL to determine their pKa values.[35]

AML and VAL have been formulated in a fixed-dose combination where approximately 80–90% of patients receiving this combination achieved a response, defined as a mean sitting diastolic blood pressure <90 mm Hg or at least 10 mm Hg reduction from baseline.[36] The assay of this drug combination was the subject of few analytical reports. These reports recommended spectrophotometric,[37] thin layer chromatography (TLC),[38,39] and HPLC[39,40] procedures. LC-MS/MS was applied for the simultaneous quantitation of the two drugs in human plasma.[41] No attempts have yet been made to assay this drug combination by a fluorescence-based method. Spectrofluorimetric methods have found many applications in the field of drug analysis. The principal advantages of these methods lie in the improved sensitivity and

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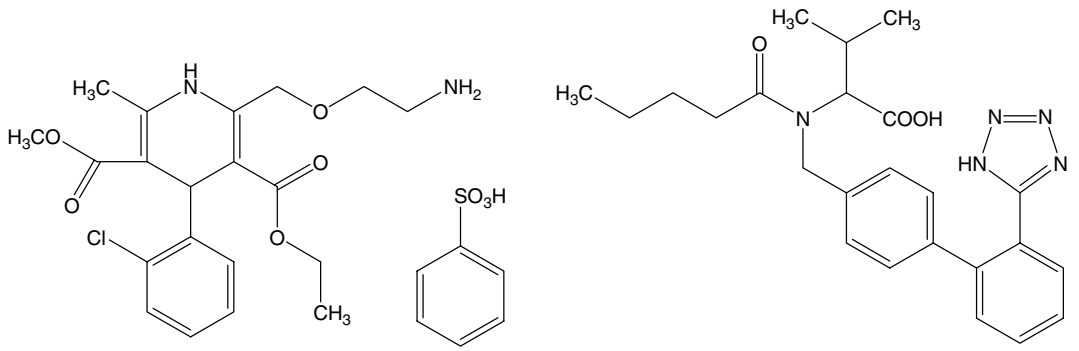

Figure 1. Chemical structures of amlodipine besylate (AML) and valsartan (VAL).

selectivity, in addition to the significant economic advantages over other sophisticated instrumental techniques. The aim of this work is to develop a simple yet sensitive and selective spectrofluorimetric method for the simultaneous determination of AML and VAL in their combined dosage form.

Experimental

Apparatus

Fluorescence measurements were carried out using a Shimadzu (Kyoto, Japan) RF-1501 version 3.0 spectrofluorophotometer equipped with a 150 W xenon lamp and 1 cm quartz cells.

Materials

AML was kindly supplied by Pfizer Egypt S.A.E. (Cairo, Egypt) and VAL was kindly provided by Novartis Pharma S.A.E. (Cairo, Egypt). Methanol and glacial acetic acid (99%) were of analytical-reagent grade. Aqueous solutions were prepared using high purity distilled water. Pharmaceutical preparation containing both drugs is Exforge® tablets (BN. Y0001/S0009) labelled to contain 10 mg AML and 160 mg VAL. The preparation is manufactured by Novartis Pharma (Basel, Switzerland) and it was purchased from the local market.

General procedure

AML stock solution (200 μg mL^{-1}) and VAL stock solution (160 μg mL^{-1}) were prepared in methanol. AML working solution (40 μg mL^{-1}) and VAL working solution (8 μg mL^{-1}) were prepared in methanol by dilution of appropriate volumes of the corresponding stock solutions. Stock and working solutions were stable for at least two weeks when stored refrigerated at 4 °C. Aqueous 0.1 M acetic acid solution was prepared and used in the study.

Standard solutions for the calibration graphs were prepared by dilution of accurate volumes of AML and VAL working solutions to reach the concentration ranges mentioned in Table 1. Dilution was made using distilled water for AML solutions and 0.1 M acetic acid for VAL solutions. Blank solutions were similarly prepared but the drugs were omitted. The fluorescence intensities for AML and VAL standard solutions were measured at the specified excitation and emission wavelengths (Table 1).

Table 1. Experimental and analytical parameters for the spectrofluorimetric determination of AML and VAL

Parameter	AML	VAL
Solvent	Distilled water	0.1 M acetic acid
$\lambda_{Ex}/\lambda_{Em}$ (nm)	360/455	245/378
Concentration range (μg mL^{-1})	0.2–3.6	0.008–0.080
Regression equation F = a + bC	F = 2.18 + 238.26C	F = 6.62 + 8025.34C
Correlation coefficient (r)	0.99978	0.99985
S_a	4.79	3.37
S_b	2.23	69.32
RSD% of the slope (S_b %)	0.94	0.86
$S_{y/x}$	7.42	4.33
LOD (μg mL^{-1})	0.025	0.0012
LOQ (μg mL^{-1})	0.082	0.0039

Assay of pharmaceutical tablets

Ten Exforge® tablets were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 10 mg AML was extracted into methanol with the aid of shaking and filtered. Dilution of the filtrate was made with methanol in order to obtain final concentration 40 μg mL^{-1} AML. Aliquots of the AML tablet solution were diluted with distilled water to obtain final concentrations within the specified range then treated as under 'General Procedure'. For the assay of VAL, a portion of the powder equivalent to 16 mg VAL was extracted into methanol with the aid of shaking and filtered. Suitable dilution of the filtrate was made to obtain final concentration 8 μg mL^{-1}. Accurate volumes of VAL tablet solution were diluted with 0.1 M acetic acid to reach the specified concentration range then treated as under 'General Procedure'.

Results and Discussion

Spectral characteristics and optimization of fluorescence measurement

An investigation of the native fluorescence characteristics of AML and VAL showed that both drugs are fluorescent in several

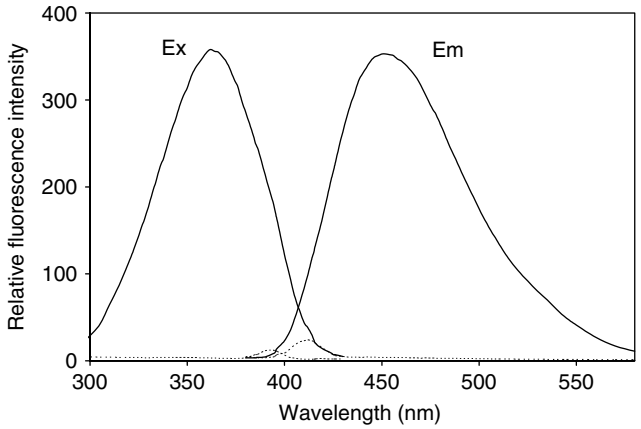

Figure 2. Excitation and emission spectra of 1.6 μg ml^{-1} AML (——) and the solvent blank (· · · ·).

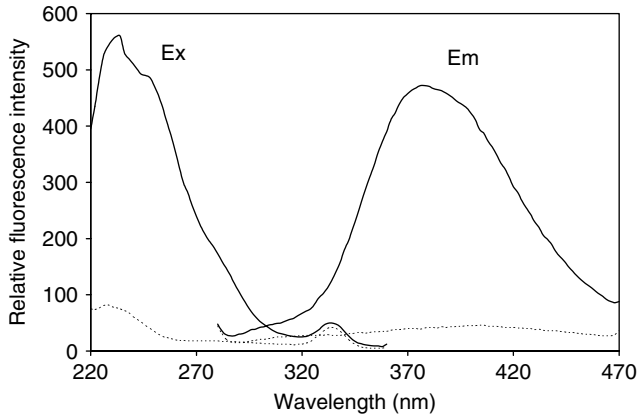

Figure 3. Excitation and emission spectra of 0.048 μg ml^{-1} VAL (——) and the solvent blank (· · · ·).

solvent media. The choice of the proper solvent was based on sensitivity of measurement, stability of fluorescence readings, and background (blank) readings. Solvents investigated include water, acid solutions (acetic, sulphuric and phosphoric acids) and alcohols such as methanol and ethanol. Generally, aqueous solutions are better than alcohols due to the high background fluorescence obtained by the use of alcohols. Water was found the best solvent for measurement of AML, while acidic solutions resulted in an obvious decline in fluorescence intensity probably due to the instability of the drug in acidic solutions. On the other hand, the fluorescence intensity of VAL in acidic medium was at least five times that in water, and among the acids tried, 0.1 M acetic acid solution was found optimum. Aqueous solutions of AML showed fluorescence at excitation and emission wavelengths 360 and 455 nm respectively, while acetic acid solutions of VAL exhibited much higher fluorescence at emission maximum 378 nm upon excitation at 245 nm (Figures 2 and 3). The fact that each compound has its specific excitation and emission maxima without overlap from the other co-formulated compound allows the selective measurement and quantification of both compounds without prior separation. The stability of AML and VAL solutions was followed by measuring the fluorescence intensity at 15-min intervals. Fluorescence intensity values were stable for at least 2 h.

Analytical performance of the method

Linearity and concentration ranges

The linearity of the proposed spectrofluorimetric procedure was evaluated by analyzing a series of different concentrations for each compound. The relative fluorescence intensities measured at the specified wavelengths were found to be proportional to the concentrations of the studied drugs. Table 1 presents the performance data and statistical parameters for the proposed method including linear regression equations, concentration ranges, correlation coefficients, standard deviations of the intercept (S_a), the slope (S_b) and the standard deviation of residuals ($S_{y/x}$). Regression analysis shows good linearity as indicated from the correlation coefficient values (>0.9997). In addition, linearity can be evaluated by calculation of the RSD% of the slope (S_b %) values which were found less than 1%.

Detection and quantification limits

The limit of detection (LOD) and the limit of quantification (LOQ), were calculated in accordance to the equations provided by the USP.[20] LOD and LOQ are defined as $3sb^{-1}$ and $10sb^{-1}$, respectively where s is the standard deviation of replicate blank responses at the working wavelengths and b is the slope of the calibration graph. Both LOD and LOQ values (Table 1) confirm the sensitivity of the proposed spectrofluorimetric method particularly for the determination of VAL.

Precision

The within-day precision for the described method was examined at three concentration levels for each compound using three replicate determinations for each concentration within one day. Similarly, the between-day precision was tested by analyzing the same three concentrations for each compound using three replicate determinations repeated on three days. Concentrations found were calculated using the corresponding regression equations and they were satisfactory. The percentage relative standard deviation (RSD%) and percentage relative error (E_r%) did not exceed 2% indicating the high repeatability of the developed method for the estimation of AML and VAL in their bulk form (Table 2).

Accuracy

An accuracy study was carried out using standard addition technique. Analyzed samples of tablets were spiked with volumes of standard AML and VAL solutions. Recovery, SD and RSD% values of the standard addition analysis confirmed that the proposed method is sufficiently accurate to be applied to the commercial tablets without interference form the commonly added excipients (Table 2).

Selectivity

Method selectivity was examined by preparing several mixtures of the two compounds at various concentrations within the linearity ranges mentioned in Table 1. These mixtures were of different ratios both above and below the normal ratio expected in tablets. The laboratory-prepared mixtures were measured at the specified wavelengths against proper solvent blanks. The recovered concentrations, RSD%; and E_r% shown in Table 3 were satisfactory thus validating the selectivity, precision and accuracy of the proposed method and demonstrating its capability to resolve and quantify AML and VAL in different ratios.

Table 2. Precision and accuracy for the determination of AML and VAL in bulk form using the proposed spectrofluorimetric method

Precision

Analyte	Nominal value (μg mL^{-1})	Within-day			Between-day		
		Found ± SD[a] (μg mL^{-1})	RSD(%)[b]	E_r(%)[c]	Found ± SD[a] (μg mL^{-1})	RSD(%)[b]	E_r(%)[c]
AML	0.8	0.7897 ± 0.0064	0.81	−1.29	0.7930 ± 0.0128	1.61	−0.88
	1.6	1.6028 ± 0.0168	1.05	0.18	1.5897 ± 0.0227	1.43	−0.64
	3.2	3.1712 ± 0.0304	0.96	−0.90	3.2290 ± 0.0428	1.33	0.91
VAL	0.016	0.01614 ± 0.00018	1.12	0.88	0.01618 ± 0.00020	1.24	1.13
	0.048	0.04721 ± 0.00037	0.78	−1.65	0.04763 ± 0.00044	0.92	−0.77
	0.064	0.06357 ± 0.00056	0.88	−0.67	0.06325 ± 0.00089	1.41	−1.17

Accuracy

Analyte	% Recovery ± SD[a]	RSD %fnb
AML	98.96 ± 0.91	0.92
VAL	99.27 ± 0.94	0.95

[a] Mean ± standard deviation for three determinations.
[b] % Relative standard deviation.
[c] % Relative error.

Table 3. Determination of AML – VAL laboratory-prepared mixtures using the proposed spectrofluorimetric method

Nominal value (μg mL^{-1})		Found ± SD[a] (μg mL^{-1})			
AML	VAL	AML	VAL	RSD(%)[b]	E_r(%)[c]
1.6	1.6	1.605 ± 0.003		0.187	0.313
1.6	3.2	1.605 ± 0.017		1.059	0.313
0.8	4.0	0.775 ± 0.004		0.516	−3.125
0.4	4.0	0.399 ± 0.002		0.501	−0.250
0.4	6.4	0.408 ± 0.002		0.490	2.000
0.8	16.0	0.788 ± 0.003		0.381	−1.500
0.4	16.0	0.407 ± 0.002		0.491	1.750
0.08	**0.08**		0.0804 ± 0.0006	0.746	0.500
0.16	**0.08**		0.0809 ± 0.0013	1.607	1.125
0.24	**0.048**		0.0469 ± 0.0003	0.640	−2.292
0.64	**0.064**		0.0645 ± 0.0007	1.085	0.781
0.32	**0.016**		0.0155 ± 0.0002	1.290	−3.125

[a] Mean ± standard deviation for three determinations.
[b] % Relative standard deviation.
[c] % Relative error.

Robustness

Robustness was examined by evaluating the influence of small variations in the experimental conditions such as working excitation wavelengths (±3 nm), working emission wavelengths (±5 nm), and concentration of acetic acid solution which is used as medium for VAL measurement (±0.01 M). These variations did not have any significant effect on the measured responses. RSD% for the measured fluorescence intensity after the studied variations did not exceed 2%.

Analysis of pharmaceutical tablets

The developed spectrofluorimetric procedure was applied for the assay of this drug combination in the pharmaceutical formulation available in the local market (Exforge® tablets). The active ingredients were extracted with methanol then aliquots were diluted with the specific working solvent for each compound prior to the fluorescence measurement. The studied drugs were directly measured without any interference from the inactive ingredients. The assay results revealed satisfactory accuracy and precision as indicated from % recovery, SD and RSD% values (Table 4).

A reference derivative spectrophotometric method[37] was adopted for the assay of the two drugs in their commercial product. In the Student's *t*- and the variance ratio *F*-tests, the calculated values did not exceed the theoretical ones at the 95% confidence level which indicated that there were no significant differences between the proposed and the reference methods with regard to the mean and SD values (Table 4).

Conclusions

In this study, a direct, simple, and sensitive spectrofluorimetric procedure was developed for the simultaneous analysis of the two antihypertensive drugs; amlodipine besylate and valsartan. Reviewing the literature exposed that there were no reports for the use of a fluorescence-based method for the assay of this mixture. The proposed method combines the rapidness and simplicity advantages of traditional spectrometric methods together with other important analytical merits, such as sensitivity and selectivity. The proposed method does not require elaborate treatment or sophisticated experimental set-up usually associated with HPLC methods of analysis. Moreover, simplicity was illustrated by the minimum requirement of chemicals and solvents since methanol was the only organic solvent used in the procedure, and final measurement of both drugs was in aqueous media. This suggests that the proposed method is cost-effective and environmentally friendly. Reliability was guaranteed by testing the various validation parameters of the method and the successful application to the drug combination tablets without prior separation or excipient interference.

Table 4. Application of the proposed spectrofluorimetric method to the analysis of AML and VAL in Exforge® tablets

	Spectrofluorimetry		Reference method (Ref 37)	
	AML	VAL	AML	VAL
% Recovery ± SD[a]	98.85 ± 0.76	99.38 ± 0.98	99.12 ± 1.24	98.93 ± 1.19
RSD %[b]	0.77	0.99	1.25	1.20
t	0.41	0.65		
F	2.66	1.48		

[a] Mean ± standard deviation for five determinations.
[b] % Relative standard deviation.
Theoretical values for t and F at $P = 0.05$ are 2.31 and 6.39, respectively.

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