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Steady-state and synchronous spectrofluorimetric methods for simultaneous determination of aliskiren hemifumarate and amlodipine besylate in dosage forms

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ABSTRACT: Aliskiren hemifumarate (ALS) and amlodipine besylate (AML) were simultaneously determined by two different spectrofluorimetric techniques. The first technique depends on direct measurement of the steady-state fluorescence intensities of ALS and AML at 313 nm and 452 nm upon excitation at 290 and 375 nm, respectively, in a solvent composed of methanol and water (10: 90, v/v) . The second technique utilizes synchronous fluorimetric quantitative screening of the emission spectra of ALS and AML at 272 and 366 nm, respectively using $\Delta\lambda$ of 97 nm. Effects of different solvents and surfactants on relative fluorescence intensity were studied. The method was validated according to ICH guidelines. Linearity, accuracy and precision were found to be satisfactory in both techniques over the concentration ranges of 1–15 and 0.4–4 μg/mL for ALS and AML, respectively. In the first technique, limit of detection and limit of quantification were estimated and found to be 0.256 and 0.776 μg/mL for ALS as well as 0.067 and 0.204 μg/mL for AML, respectively. Also, limit of detection and limit of quantification were calculated in the synchronous method and found to be 0.293 and 0.887 μg/mL for ALS as well as 0.034 and 0.103 μg/mL for AML, respectively. The methods were successfully applied for the determination of the two drugs in their co-formulated tablets. The results were compared statistically with reference methods and no significant difference was found. The developed methods are rapid, sensitive, inexpensive and accurate for the quality control and routine analysis of the cited drugs in bulk and in pharmaceutical preparations without pre-separation. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: Spectrofluorimetry; synchronous; aliskiren hemifumarate; amlodipine besylate; pharmaceutical preparations; validation

Introduction

Aliskiren hemifumarate (ALS) (2(S), 4(S), 5(S), 7(S)-N-(2-carbamoyl-2-methylpropyl)-5-amino-4-hydroxy 2,7 diisopropyl-8-(4-methoxy-3-(3-methoxypropoxy)phenyl) octanamide hemifumarate) (Fig. 1a), is the first orally active direct renin inhibitor. It may provide higher protection from hypertension (1). In addition, its efficacy to decrease systolic and diastolic blood pressure could be compared to other first-line antihypertensive agents. Additional advantages can be achieved when it is used in combination with other antihypertensive drugs (1). Amlodipine besylate (AML) (3-Ethyl 5-methyl (4RS)-2-((2-aminoethoxy) methyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzenesulfonate) (Fig. 1b), is a long-acting dihydropyridine calcium channel blocker that inhibits the influx of calcium ions into vascular and cardiac muscles (2). Amlodipine acts directly on vascular smooth muscles and reduces peripheral vascular resistance and blood pressure (3). Combination therapy of ALS and AML proved to be effective and well tolerated in treatment of hypertensive patients (4).

A literature survey revealed liquid chromatography (LC) (5–8), capillary electrophoresis (9), ultraviolet-spectrophotometric (10,11) and spectrofluorimetric (12,13) methods have been reported for estimation of ALS alone or in combination with other antihypertensive agents. In addition, LC (14–16), LC with tandem mass spectrometry (2), capillary electrophoresis (17), ultraviolet-spectrophotometric (18–20) and spectrofluorimetric (21,22) methods were reported for estimation of AML alone or in combination with other agents. To the best of our knowledge, there are no reported direct or synchronous spectrofluorimetric methods for determination of ALS either alone or in combination with AML without the need for derivatization methodologies. Thus, the aim of the present study was to develop validated, sensitive, simple, rapid, inexpensive and precise spectrofluorimetric procedures for simultaneous analysis of ALS and AML in bulk drug samples and in their combined dosage formulations. The developed methods are suitable for quality control and routine analysis of ALS and AML in their combined pharmaceutical preparations.

Experimental

Materials and reagents

All the chemicals used in the present studies were of analytical reagent grade and the solvents were of HPLC grade. The working

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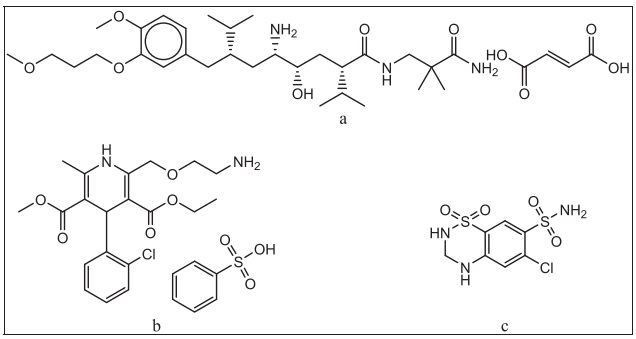

Figure 1. Chemical structures of aliskiren hemifumarate (a), amlodipine besylate (b) and hydrochlorothiazide (c).

standard of ALS (certified to contain 99.51%) was kindly supplied by Novartis Pharmaceuticals Corporation (East Hanover, NJ, USA). AML (certified to contain 99.75%) was kindly supplied by Global Nabi Pharmaceuticals Co. (Giza, Egypt). Hydrochlorothiazide (HCZ, (Fig. 1c); certified to contain 99.93%) was supplied by AstraZeneca (Cairo, Egypt, under license of AstraZeneca, Sweden). Tekamlo® 150/10 mg tablets labeled to contain 165.8 mg ALS and 13.9 mg AML per each tablet were purchased from commercial sources in the local market. Methanol, sodium dodecyl sulfate (SDS), cetyl trimethyl ammonium bromide (CTAB), hydroxymethyl cellulose (HMC) and Triton® X-100 were obtained from Sigma-Aldrich (St. Louis, MO, USA). β-Cyclodextrin (β-CD) (TCI, Tokyo Kasei, Japan). Orthophosphoric acid 85% and hydrochloric acid (Fisher Scientific, Fair Lawn, NJ, USA) were used. Sodium hydroxide and sodium dihydrogen phosphate were purchased from J.T. Baker (Phillipsburg, NJ, USA). Phosphate buffer (0.2 M, pH 4) solution was freshly prepared. SDS, CTAB, β-CD, HMC and Triton® X-100 were prepared as 1% w/v aqueous solutions. Also, 0.1 N NaOH and 0.1 N HCl were prepared.

Instruments

LS 55 Fluorescence Spectrometer, 120 V (PerkinElmer, Waltham, MA, USA), equipped with a high energy pulsed xenon source for excitation, variable slit and holographic gratings was used. Fluorescence spectra and measurements were recorded using PekinElmer FL WinLab™ software. A 1 cm quartz cell was used. A VWR SympHoly (SB20) pH meter (Thermo Orion, Beverly, MA, USA) was used for pH measurements. Deionized water was prepared using a Barnstead NANO pure Dlamond Analytical ultrapure water system (Thermo Fischer Scientific, Waltham, MA, USA).

Standard stock solutions preparation

Ten mg of ALS, AML and HCZ were accurately weighed and transferred separately into 10 mL volumetric flasks. Then, they were dissolved and made up to volume with methanol to give concentrations of 1000 μg/mL for each. Further dilutions were made separately using the same solvent to prepare 100 μg/mL of AML and HCZ.

Procedure

Linearity and construction of calibration graphs. Aliquots of ALS and AML standard stock solutions were separately transferred into two series of 10 mL Fisherbrand disposable tubes (Fischer Scientific, Waltham, MA, USA) to give final concentrations of 1–15 and 0.4–4 μg/mL for ALS and AML, respectively, then completed to 500 μl with methanol. Finally, 4.5 mL deionized water was added to each tube to reach a final volume of 5 mL, the contents were mixed well. Relative fluorescence intensity (RFI) was measured in two ways. In the first technique, it was measured directly against a solvent blank at 313 and 452 nm upon excitation at 290 and 375 nm for ALS and AML, respectively. The excitation and emission slit width of 4.5 nm were used in the case of ALS as well as 8 nm in the case of AML (Fig. 2). In the second technique, RFI of the synchronous spectrofluorimetric spectra was measured against a solvent blank using $\Delta\lambda$ of 97 nm at 272 and 366 nm for ALS and AML, respectively (Fig. 3). The excitation and emission slit width were 8 nm. RFI was plotted versus the corresponding final drug concentration of ALS and AML (μg/mL) to obtain the calibration graphs and regression equations.

Analysis of aliskiren hemifumarate and amlodipine besylate laboratory prepared mixtures. Aliquots of ALS and AML standard stock solutions were accurately transferred into a series of 10 mL Fisherbrand disposable tubes to give final concentrations of 4.8–14.4 and 0.4–1.2 μg/mL for ALS and AML, respectively. The procedure described under "Linearity and construction of calibration graphs" was then applied. The recovery percentage was calculated using the corresponding regression equation.

Sample preparation. Twenty Tekamlo® tablets were weighed and finely powdered. A portion of the powder equivalent to ALS (50.00 mg) and AML (4.19 mg) was introduced into a 50 mL volumetric flask and sonicated with 30 mL methanol for 15 min, and then completed to the mark with methanol. The solution was filtered through a Whatman filter paper. The procedure described under "Linearity and construction of calibration graphs" was then applied. The recovery percentage was calculated for each drug using the corresponding regression equation. A standard addition technique was applied and the concentrations of the examined drugs were calculated using the corresponding regression equation.

Validation

The suggested analytical method was validated according to ICH guidelines (23) with respect to certain parameters such as linearity, limit of detection, limit of quantification, accuracy, precision, specificity and stability.

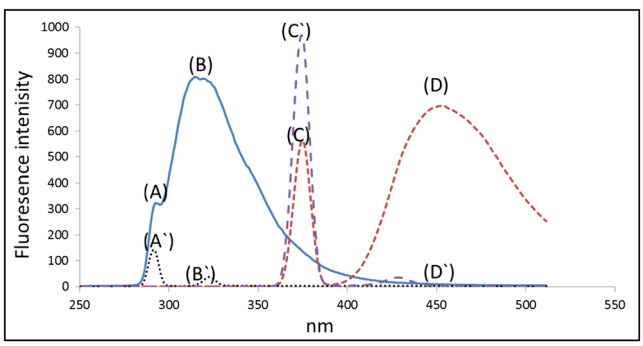

Figure 2. Excitation (A,C) and conventional fluorescence spectra of (B,D) of aliskiren hemifumarate and amlodipine besylate, respectively (aliskiren hemifumarate: 12 μg/mL and amlodipine besylate: 3 μg/mL) in a solvent composed of methanol and water (10: 90, v/v) and (A',B',C',D') for a blank solvent composed of methanol and water (10: 90, v/v). Where (A,C, A',C`) are the excitation spectra and (B,D,B',D') are the emission spectra.

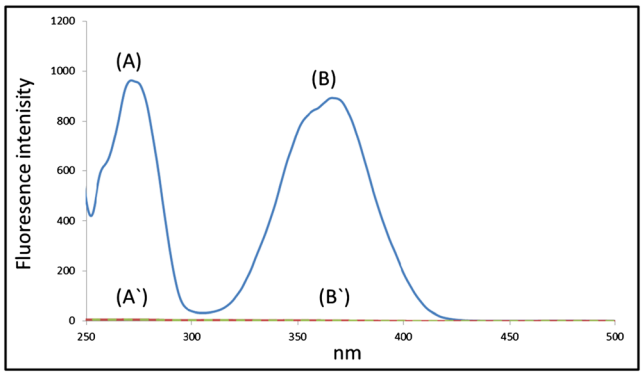

Figure 3. Synchronous fluorescence spectra of (A) aliskiren hemifumarate (12 μg/mL), (B) amlodipine besylate (3 μg/mL) in a solvent composed of methanol and water (10: 90, v/v) and (A′,B′) synchronous fluorescence spectrum for a solvent blank composed of methanol and water (10: 90, v/v) using the same parameters.

Linearity. The linearity of the method was established by spiking a series of standard mixtures in the range 1–15 and 0.4–4 μg/mL of ALS and AML, respectively. The procedure described under "Linearity and construction of calibration graphs" was applied. Linear regression was assessed and standard deviation of slope (S_b), standard deviation of intercept (S_a), correlation coefficient (r) and standard error (E_s) were determined.

Limit of detection and limit of quantification. LODs and LOQs were determined through the dilution method using a signal to noise (S/N) approach. LOD was considered the minimum concentration with a S/N ratio of at least 3, while LOQ was taken as the minimum concentration with a S/N ratio of at least 10 (23).

Accuracy. Accuracy was determined in terms of recovery percentage. A set of standard mixtures at six different concentration levels 4.8, 7.2, 9.6, 12, 13.2 and 14.4 μg/mL of ALS and 0.4, 0.6, 0.8, 1, 1.1 and 1.2 μg/mL of AML were prepared. The procedure described under "Linearity and construction of calibration graphs" was then applied. The recovery percentage of ALS and AML was calculated.

Precision. Method precision was determined both in terms of repeatability (intraday reproducibility) and intermediate precision (interday reproducibility) using three different concentration levels 2.0, 6.0 and 12.0 μg/mL of ALS and 0.6, 1.8 and 3.4 μg/mL of AML.

Specificity. Specificity was examined by analyzing ALS and AML in their laboratory prepared mixtures and pharmaceutical dosage forms to detect any interference caused by the combined drugs present in dosage formulations or the co-formulated excipients. In addition, specificity was checked by analyzing 12 μg/mL ALS and 0.5 μg/mL AML in their triple mixture with 0.5, 1 and 2 μg/mL of HCZ. The recovery percentage and standard deviation were calculated.

Stability. The stability studies of ALS and AML samples were carried out over a period of 72 h at 2–8 °C (refrigerator) and standard stock solutions for 1 month at 2–8 °C.

Results and discussion

The goal of the present investigation was to develop two validated, sensitive, simple and rapid spectrofluorimetric methods for simultaneous determination and quantification of ALS and AML in pharmaceutical preparations. In addition, many trials were attempted to enhance the intensity of the emission bands through selection of proper solvent and addition of surfactants at concentrations above their critical micellar concentration in order to perform micelles or reversed micelles able to stabilize the exited singlet states and delay the decay process. The fluorescence properties of ALS was studied in various solvents and micellar media and a solvent composed of methanol and water (10: 90, v/v) was chosen as none of the studied media caused significant enhancement in RFI of ALS (Figs 4 and 5).

Optimization of experimental conditions

Effect of different organized media. The conventional fluorescence properties of ALS in various micellar media were studied using anionic surfactant (SDS), cationic surfactant (CTAB) and non-ionic surfactant (Triton® X-100) as well as different macromolecules, such as β-CD and HMC. ALS has a pKa above 9 so it will be positively charged under acidic conditions obtained by adding phosphate buffer (pH 4). None of the examined surfactants showed significant enhancement in RFI of ALS (Fig. 4).

Effect of diluting solvent. For the steady-state conventional spectrofluorimetric procedure, the influence of different diluting solvents on RFI of ALS was investigated using water, methanol and phosphate buffer solution (PBS) at pH 4, 0.1 N HCl and 0.1 N NaOH to give final solutions containing 10% v/v methanol in each. Water was chosen as the diluting solvent as it showed a good RFI compared to methanol but it is friendlier to the environment and with fewer blank readings (Fig. 5).

Selection of Δλ in the synchronous spectrofluorimetric method. To separate entirely synchronous fluorescence peaks of ALS and AML, the synchronous fluorescence spectra of the mixtures were recorded using different Δλ. It was found that Δλ of 97 nm is suitable for simultaneous determination of ALS and AML in their medicinally recommended ratio of (12: 1). As shown in Fig. 3, the maximum synchronous fluorescence peaks of ALS and AML were at 272 and 366 nm, respectively; no significant interference would occur in their medicinally recommended ratio.

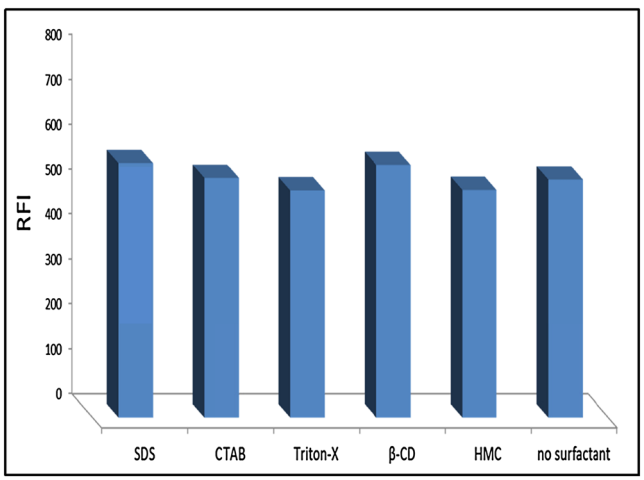

Figure 4. Effect of type of surfactant (1 mL of 1% solution for each) on the conventional RFI of aliskiren hemifumarate (8 μg/mL). β-CD, β-cyclodextrin; CTAB, cetyl trimethyl ammonium bromide; HMC, hydroxymethyl cellulose; RFI, relative fluorescence intensity; SDS, sodium dodecyl sulfate.

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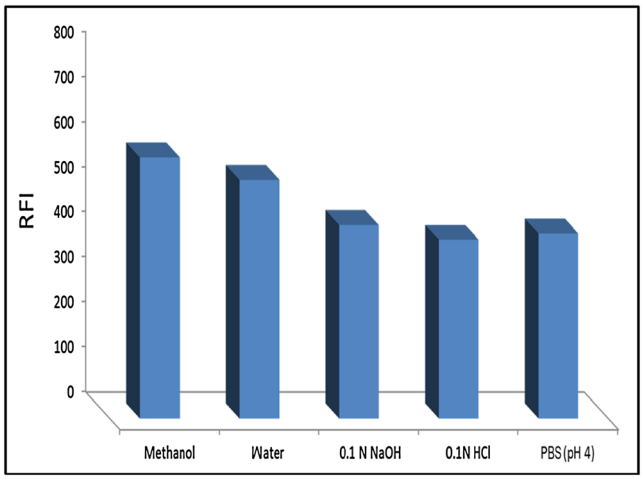

Figure 5. Effect of different diluting solvents on the conventional RFI of aliskiren hemifumarate (8 μg/mL). PBS, phosphate-buffer solution; RFI, relative fluorescence intensity.

Validation of the methods

Linearity and range. A linear relationship between the RFI of ALS and AML versus their concentrations was obtained. The regression equation $y = bC \pm a$ for each drug was also computed. In this study, five concentrations for ALS and AML were used. The linearity of the calibration curves were validated by the high value of correlation coefficients (r) of the regression equation, small values of the standard deviation of residuals ($S_{y/x}$) and small value of the percentage relative error (Table 1). The analytical data of the calibration curves, including standard deviations for the slope and intercept (S_b, S_a), are summarized in Table 1. These data indicated the linearity of the calibration graphs.

Limit of detection and limit of quantification. LODs and LOQs for each drug were calculated. Results are given in Table 1.

Accuracy. Accuracy of the results was investigated by calculating recovery percentage of six different concentrations of the two drugs analyzed by the proposed spectrofluorimetric methods. The results obtained, including the mean of the recovery percentage and standard deviations, are displayed in Table 1. To prove the accuracy and utility of the proposed method, the results of the assay of ALS and AML with the proposed spectrofluorimetric methods were compared with those of the reference methods (6,24). Statistical analysis of the results using Student's t-test and variance ratio (F-test) revealed no significant difference between the performance of the developed methods and the reference methods regarding the accuracy and precision, respectively (Table 2). The reference method (6) and the pharmacopoeial method (24) depend on the analysis of ALS and AML, respectively, using reverse phase HPLC.

Precision. Repeatability (intraday, $n = 3$) and intermediate precision (interday, $n = 3$) were checked using three different concentrations at low, medium and high level of the standard curve. Percentage of relative standard deviation was calculated to check the precision of the methods (Table 3).

Specificity. The specificity of the methods was investigated by observing any interference encountered from the excipients of the tablets. It was shown that these compounds do not interfere with the proposed methods (Table 1). It was also found that ALS and AML do not interfere with each other either in their laboratory prepared mixtures or in their co-formulated tablets (Table 1). Moreover, the steady-state method showed better results and higher tolerance in presence of up to 1 μg/mL HCZ for determination of ALS and AML in comparison with the synchronous method (Table 1). Therefore, the steady-state method is more reliable to be

Table 1. Performance data and results of the proposed spectrofluorimetric methods for the simultaneous determination of ALS and AML

Item	Conventional fluorescence		Synchronous fluorescence	
	ALS	AML	ALS	AML
Linearity range (μg/mL)	1–15	0.4–4	1–15	0.4–4
LOD (μg/mL)	0.256	0.067	0.293	0.034
LOQ (μg/mL)	0.776	0.204	0.887	0.103
Regression equation (Y)[a]: Slope (b)	63.679	241.398	60.459	233.118
Standard deviation of slope (S_b)	0.548	2.114	0.595	1.031
Intercept (a)	7.1382	0.4170	28.9113	− 0.2143
Standard deviation of intercept (S_a)	4.942	4.928	5.362	2.403
Regression coefficient (r^2)	0.9999	0.9998	0.9999	0.9999
Standard deviation of residuals ($S_{y/x}$)	5.937	6.480	6.442	3.159
% Error	1.292	0.448	1.378	0.467
Results	(Recovery percentage ± SD)			
Drug in bulk	101.33 ± 0.91	99.81 ± 0.84	100.74 ± 0.88	100.68 ± 0.72
ALS and AML synthetic mixtures	100.14 ± 1.90	100.46 ± 1.30	99.67 ± 1.05	99.49 ± 0.54
ALS and AML ternary mixtures with HCZ	100.72 ± 1.58	100.02 ± 0.35	95.68 ± 1.39	99.86 ± 0.77
TekamLo® 150/10 mg tablets	97.14 ± 0.29	96.22 ± 2.02	96.98 ± 1.28	97.81 ± 1.37
Drug spiked to dosage form	100.74 ± 1.03	100.63 ± 1.03	100.31 ± 1.08	100.78 ± 1.46

ALS, aliskiren hemifumarate; AML, amlodipine besylate.
[a] $Y = a + bC$, where C is the concentration in μg/mL and Y is the peak area.

Table 2. Statistical comparison between analysis results of pure samples of the studied drugs using the proposed spectrofluorimetric methods and those of the reference methods

Statistical term	ALS			AML		
	Reported method (6)	Conventional fluorescence	Synchronous fluorescence	Reported method (24)	Conventional fluorescence	Synchronous fluorescence
Mean	101.10	101.33	100.74	99.90	99.81	100.68
± SD	1.64	0.91	0.88	1.59	0.84	0.72
± SE	0.73	0.37	0.36	0.71	0.34	0.29
%RSD	1.62	0.90	0.87	1.59	0.84	0.72
n	5	6	6	5	6	6
Variance *V*	2.69	0.83	0.77	2.53	0.71	0.52
Student's *t*-test		0.783 (1.833[a])	1.003 (1.833[a])		0.180 (1.833[a])	1.312 (1.833[a])
F-test		3.241 (6.260[a])	3.494 (6.260[a])		3.563 (6.260[a])	4.865 (6.260[a])

[a]Figures in parentheses are the theoretical *t* and *F* values at ($P = 0.05$) and *n* is the number of experiments.

Table 3. Precision data of the proposed spectrofluorimetric methods for determination of ALS and AML

	Intra-days assay		Inter-days assay	
	Conventional fluorescence Found ± %RSD[a]	Synchronous fluorescence Found ± %RSD[a]	Conventional fluorescence Found ± %RSD[a]	Synchronous fluorescence Found ± %RSD[a]
Concentration of ALS (μg/mL)				
2	1.997 ± 0.79	1.959 ± 2.97	2.004 ± 1.18	1.967 ± 1.90
6	5.975 ± 1.35	6.006 ± 1.26	5.996 ± 1.32	5.942 ± 1.74
12	12.021 ± 0.72	12.092 ± 1.54	11.955 ± 1.13	12.056 ± 1.20
Concentration of AML (μg/mL)				
0.6	0.609 ± 1.42	0.591 ± 1.51	0.604 ± 1.55	0.601 ± 2.09
1.8	1.797 ± 0.96	1.813 ± 0.90	1.814 ± 1.13	1.835 ± 1.26
3.4	3.397 ± 0.46	3.394 ± 0.58	3.419 ± 0.97	3.396 ± 0.41

ALS, aliskiren hemifumarate; AML, amlodipine besylate.
[a]All results are the average of three determinations

used for simultaneous determination of ALS and AML in presence of HCZ specifically in the medicinally recommended ratio of (12: 0.5: 1), respectively, in their co-formulated tablets, Amturnide® (Table 1).

Stability. Results from the stability studies of standard stock solutions indicated that they were stable for 1 month at 2–8 °C with satisfactory recovery percentage of more than 98%. These solutions can therefore be used during this interval of time without the results being affected.

Conclusion

Rapid, sensitive and simple spectrofluorimetric methods were developed and validated for the simultaneous determination of ALS and AML in bulk and in dosage forms. The proposed methods have many advantages regarding analysis time, sensitivity and cost compared with those of the previously reported methods. Moreover, lower values for LOD and LOQ could be obtained easily by controlling the slit width of excitation and emission, which could allow determination of these drugs separately in plasma using the synchronous spectrofluorimetric technique. On the other hand, the direct steady-state method showed good tolerance and could be applied to the determination of ALS and AML in their co-formulated tablets with HCZ. Finally, the proposed methods can be used for the quality control of the cited medications in ordinary laboratories.

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