Journal of Applied Pharmaceutical Science

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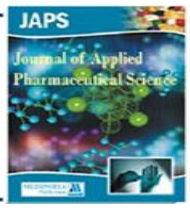

ISSN: 2231-3354
Received on: 17-04-2012
Revised on: 19-04-2012
Accepted on: 08-06-2012
DOI: 10.7324/JAPS.2012.2622

Spectrophotometric determination of amlodipine and nicardipine in pharmaceutical formulations via binary complex formation with eosin Y

Sayed M. Derayea, Hassan F. Askal, Osama H. Abdel-Megeed and Mohamed A. El Hamd

Sayed M. Derayea
Analytical Chemistry Department, Faculty of Pharmacy, Minia University, Minia 61519, Egypt.

Hassan F. Askal
Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt.

Osama H. Abdel-Megeed
Pharmaceutical Chemistry Department, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia.

Mohamed A. El Hamd
Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Al-Azhar University, Assiut 71524, Egypt.

For Correspondence
Sayed M. Derayea
Analytical Chemistry Department, Faculty of Pharmacy, Minia University, Minia 61519, Egypt.

ABSTRACT

A validated simple and selective spectrophotometric method was developed for the selective determination of amlodipine and nicardipine in bulk powders and pharmaceutical formulation. The proposed method was based on the formulation of a binary complex between either of the studied drugs and eosin Y in aqueous buffered medium. The surfactant, methylcellulose, was added to enhance the solubility of the formed complex. The binary complexes showed absorption maxima at 549 nm. The different experimental parameters affecting the development of stability of the colors were studied and optimized. Under the optimum reaction conditions, linear relationship with good correlation coefficients (0.9981 and 0.9995) were found between the absorbances and the concentrations of amlodipine and nicardipine respectively in the range of 5-60 μg/ml for both drugs. The limits of detection were 1.8 and 1.2 μg/ml while the limits of quantitation were 6.0 and 3.6 μg/ml for both drugs respectively. The analytical parameters were fully validated and results were satisfactory. No interference was observed from the excipients that are commonly present in pharmaceutical formulations. The proposed method was successfully applied to the analysis of the cited drugs in some pharmaceutical formulations. The mean percentage recoveries were 100.04 ± 0.83 and 99.98 ± 0.80. The results obtained are reproducible with a coefficient of variation less than 2% and were in good agreement with those obtained using the reference methods.

Keywords: Amlodipine; nicardipine; spectrophotometry; eosin; pharmaceutical analysis.

INTRODUCTION

Amlodipine (AML) and nicardipine (NIC) are calcium channel blockers belong to 1,4-dihydropyridines (Fig. 1). They are primarly used in the treatments of cardiovascular diseases such as hypertension, angina and some forms of cardiac arrhythmias. .(Sweetman 2009).

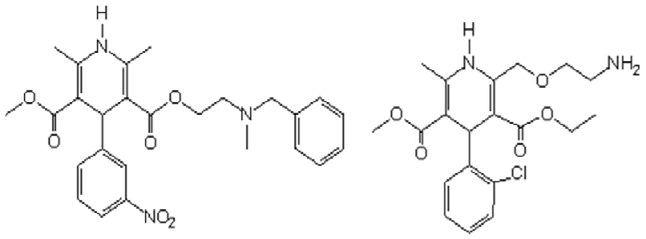

Fig. 1: Chemical structures of nicardipine (left) and amlodipine (right).

Several analytical methods have been developed for the determination of AML and NIC in their bulk, pharmaceutical formulations and/or biological fluids. Among the reported analytical methods for AML are spectrofluorometric methods(Abdel-Wadood *et al.* 2008), TLC(Argekar and Powar 2000; Rahman *et al.* 2004), HPTLC(Meyyanathan and Suresh 2005), HPLC(Patil *et al.* ; Tatar and Atmaca 2001; Bahrami and Mirzaeei 2004; Naidu *et al.* 2005; Chaudhari *et al.* 2007; Mohammadi *et al.* 2007), SPC-HPLC(Wei *et al.* 2009), HPLC-MS(Streel *et al.* 2002; Baranda *et al.* 2005), voltammetry(Gazy 2004), and CE(Wang *et al.* 2007). The reported methods for NIC include; spectrofluorometric methods(Al-Ghannam and Al-Olyan 2008; Walash *et al.*), SPC-HPLC(Wei *et al.* 2009), HPLC-MS(Baranda *et al.* 2005; Qi *et al.* 2006), UPLC-MS(Kalovidouris *et al.* 2006; Lei *et al.* 2009), and Micellar electrokinetic chromatography(Martinez *et al.* 1999),

Due to the inherent simplicity of spectrophotometric methods, economic advantages, and availability of their instruments in most quality control laboratories, these methods are the most widely used techniques in pharmaceutical analysis.

However, few spectrophotometric methods have been described for the determination of AML (Sridhar *et al.* 1997; Rahman and Azmi 2000; Basavaiah *et al.* 2003; Rahman and Nasrul Hoda 2003) and NIC(Huang and Li 1990; Al-Ghannam and Al-Olyan 2009).

In this work, a simple, sensitive, and accurate non extractive spectrophotometric method was described for the determination of AML and NIC. This method is based on the reaction of the amino group in AML or NIC, with acidic dye, eosin Y, to form ion pair associate in the presence of Mcllvaine buffer. The water solubility of the formed ion pair is enhanced by the addition of non ionic surfactant, methyl cellulose. As a result the extraction step was omitted. The absorbance of the developed color is measured at 549 nm. The method, after its full optimization and validation, was adopted for the determination of DSL in its pharmaceutical formulations.

EXPERIMENTAL

Apparatus

UV-1601 PC (Shimadzu, Kyoto, Japan ultraviolet–visible spectrophotometers with matched 1 cm quartz cells was used for all measurements. Jennway® 6505 (London, U.K.), Ultraviolet/Visible spectrophotometer.

Chemicals and reagents

Nicardipine hydrochloride (Global Napi/Wockhardt, Cairo, Egypt), and amlodipine besylate (T3A, Assuit, Egypt). All drugs were used as received without further purification.

Methanol, ethanol, acetone, acetonitrile, glucose, lactose, sucrose, magnesium stearate, talc, starch, gum acacia, ferric chloride, potassium ferrocyanide, sodium hydroxide, potassium hydroxide, di-sodium hydrogen phosphate and citric acid; all these chemicals were obtained from (El-Nasr Co. for pharmaceuticals and chemicals, Egypt).

Eosin Y (Merck, Darmstadt, Germany). was prepared freshly (0.020% W/V) in distilled water. Mcllvaine buffer**,** solution was prepared by mixing specific volumes of 0.2 M disodium hydrogen phosphate and 0.1 M citric acid to get the required PH values in the range of 2-6. Methyl cellulose (Prolabo, France, 1500 CPS) was prepared as 0.3% w/v by dissolving the appropriate amount in hot water (80°C) with stirring for 10 min, then chilling to 5°C for 30 min.

Solutions were prepared with double distilled water which was obtained through Nanopure II water purification system (Barnstead/ Thermolyne, Dubuque, IA, USA).

Pharmaceutical formulations

Pelcard SR® capsules (Global Napi /Wockhardt, Cairo, Egypt) is labeled to contain 50 mg nicardipine. Alkapress® tablets (Alkan ,Cairo, Egypt), Myodura® tablets (Global Napi /Wockhardt, Cairo, Egypt) and Vasonorm® tablets (Pharco, Alexandera, Egypt) are labeled to contain 10 mg amlodipine. Amlodipine® tablets (Global Napi /Wockhardt, Cairo, Egypt) and Regcor® tablets (Egyptian International Pharmaceutical Industries Co., Cairo, Egypt. (EPCO)) are labeled to contain 5 amlodipine.

Preparation of standard and sample solutions

Preparation of standard solutions

The stock standard solutions were prepared by dissolving an accurately weighed amount (25 mg) of the drug using about 15 ml of ethanol or distilled water in a 25-ml volumetric flask for AML or NIC respectively. The contents of the flask were sonicated for 5 min, then complete to 25 ml with distilled water. The working standard solutions were prepared by further dilution of the stock solution with the same solvent to obtain concentrations covering the required range.

Preparation of pharmaceutical dosage forms

An accurately weighed amount of the powdered tablets equivalent to 25 mg of the drug was transferred quantitatively into 25 mL volumetric flask. The content of the flask was sonicated for 5 min. with about 15 ml of methanol or distilled water for NIC or AML respectively. The resultant mixture was completed to volume with distilled water. The solution was filtered and the first portion of the filtrate was rejected. A portion of the filtrate was diluted quantitatively with distilled water to the required final concentration.

General recommended procedure

One milliliter of sample or standard solution was transferred into a 10-ml calibrated volumetric flask. 0.5 ml Methyl cellulose surfactant (0.3%) and 0.5 ml of Mcllvaine buffer (pH 4 for NIC and pH 3.5 for AML) was added. One milliliter of Eosin Y (3×10^{-4} M) was added. The solution was allowed to stand for 10 min at room temperature, and then completed to the mark with distilled water. The absorbance was measured at 549 nm against reagent blank treated similarly.

Determination of molar ratio of the reactions between drugs and eosin Y

Job's method of continuous variation was employed under the working condition to established the stiochiometry of the reaction; Master solutions of equimolar concentration (3×10^{-2} M) of both eosin Y and the investigated drugs were prepared. Series of 1.0 ml portions of the master solutions of eosin Y and drug were made up comprising different complimentary proportions (0:1.0, 0.1:0.9, …, 0.9:0.1, 1.0:0) in 10-ml volumetric flasks. 0.5 ml Methyl cellulose surfactant (0.3%) and 0.5 ml of Mcllvaine buffer (pH 4 for NIC and pH 3.5 for AML) was added. The reactions were allowed to stand for 10 min at room temperature. The volume was completed to 10 ml with distilled water, and the absorbance was measured at 549 nm against reagent blank treated similarly, except the drugs was omitted.

RESULTS AND DISCUSSION

Binary complexes between eosin and basic compounds have been widely used in spectrophotometric analysis of some drugs(Walash *et al.* 2007; Chen *et al.* 2008). Because of the water insolubility of the formed ion pair associate, it was usually extracted by organic solvent such as chloroform. This complicated the method is by the extraction step. In this work the water solubility of the formed complex is greatly enhanced by the addition of a surfactant. This enables the direct measurements in the aqueous solution without the need for the extraction step.

Involved reaction, and absorption spectra

The reaction involved in the present study is based on the interaction of the primary or tertiary amino groups in AML or NIC molecules respectively with eosin Y. The formed ion pair associate has absorption maxima at 549 nm as shown in Fig. 2. This color reaction was employed for the development of a novel spectrophotometric method for the determination of both drugs.

Optimization of reaction conditions

All factors affecting the reaction between the studied drugs, and eosin Y, have been studied, optimized and applied in the development of the assay procedures. These factors included; concentrations of eosin Y, pH, type and concentration of surfactant, temperature, and the diluting solvent).

An absorbance intensity of about 0.9 was obtained when the concentration of eosin Y in the final assay solution was 0.02 mg/ml (about 3 x 10^{-4} M). Higher eosin Y concentration had no effect on the reaction but result in the increase in the blank absorbance.

The influence of pH on the absorbance of the binary complex was studied over a range of 2.6 to 6 using Mcllvaine buffer. As shown in **Fig. 3,** the complex formation was greatly affected by pH of the medium. Maximum absorbance values were obtained when the pH of reaction mixtures were 3.5 and 4.0 for NIC and AML respectively.

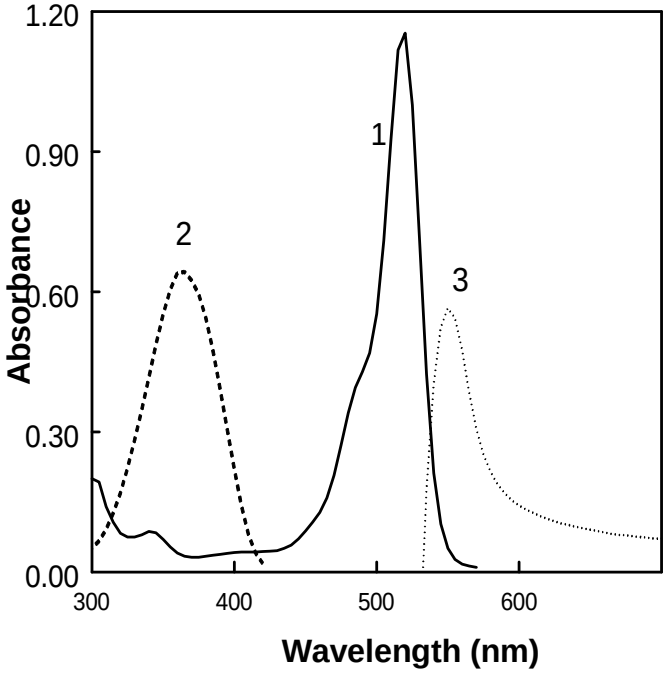

Fig. 2: Absorption spectra of 0.020 %, w/v eosin Y (1), 50 µg/ml AML (2), and their reaction product (3).

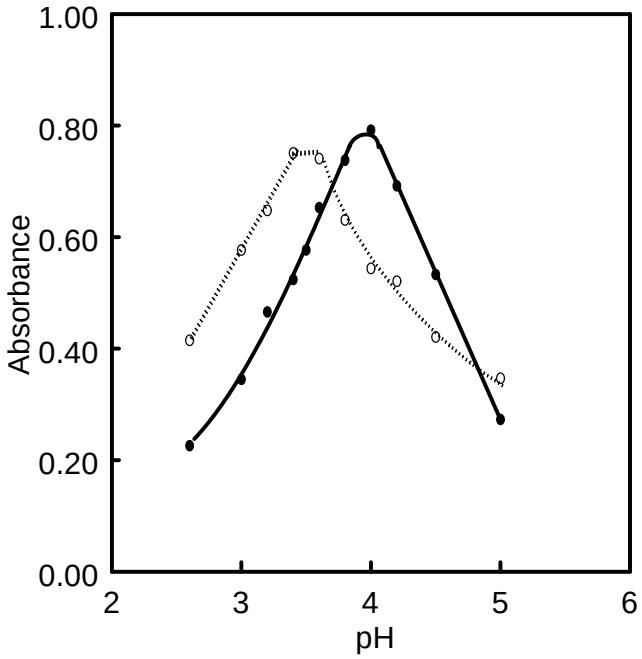

Fig. 3: Effect of pH on the absorption intensity of the reaction products of eosin Y with 50 µg/ml of NIC (-○-) and AML (-●-).

Cationic and anionic surfactants cannot be used for the enhancement of the water solubility of the formed complex. Both greatly interfere the analysis by either reacting with the dye as in the case of cationic surfactant or by preventing the formation of the colored complex as in the case of anionic surfactant. The effect of different non ionic surfactants (Fig 4) on the absorbance intensities had been tried. Methyl cellulose was found to be the most effective one in the prevent precipitation of the binary complex and with longer wavelength [Tween 80 (540 nm), Tween 20 (545 nm), Carboxymethyl cellulose (546 nm), Polyvinyl alcohol (547 nm), and Methyl cellulose (549 nm)]. The absorption intensities increase by increasing the surfactant concentration. The suitable concentration was 0.015 % and higher concentration has no effect. The most appropriate solvent for dilution was tested by using different solvents (table 1). The studied solvents included acetone, methanol, ethanol, 1-propanol and water. The highest color intensity was obtained when water was used as diluting solvent.

The reaction between the 1,4 dihydropyridine drugs and eosin Y was completed at room temperature within 5 min and stable for at least 35 min, however for more precise reading the measurements were carried out after 10 min.

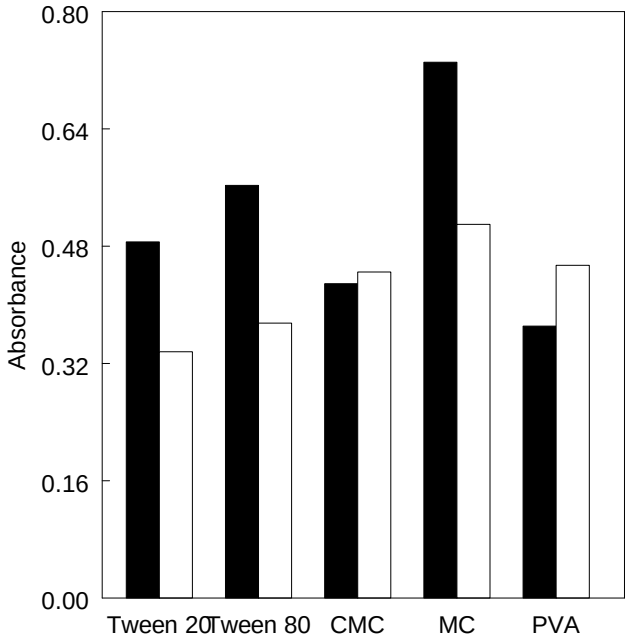

Fig 4: Effect of different surfactants on the color intensity of the reaction product of AML (-□-) and NIC (-■-) with eosin. CMC; Carboxymethyl cellulose, MC; Methyl cellulose and PVA; Polyvinyl alcohol

Table 1: Effect of solvents on the absorption intensity of the reaction products of 5 µg/ml drugs with eosin Y.

Solvent	Di-electric constant(Mandip and Babu 2006)	Absorbance *	
		NIC	AML
Water	80.5	0.612	0.524
Methanol	32.7	0.300	0.254
Ethanol	24.3	0.221	0.211
Acetone	20.7	0.133	0.121
Propan-1-ol	20.1	0.211	0.213

* The values are the mean of three determinations.

Stoichiometry and kinetics of the reaction

Job's method of continuous variation was used to establish the stoichiometry of the reaction between the investigated drugs and eosin Y. The result revealed a 1:1 ratio for drug : eosin Y (Fig. 5). Based on this ratio, a proposal of the reaction pathway can be explained. The binary complex, was probably formed via the electrostatic interaction between the basic nitrogen atom (primary or secondary amino groups in AML or NIC respectively) which is the most basic center in the drug molecules and the carboxylate anion of the dye. This might primarily occur in acidic medium as the amino group is maximally protonated.

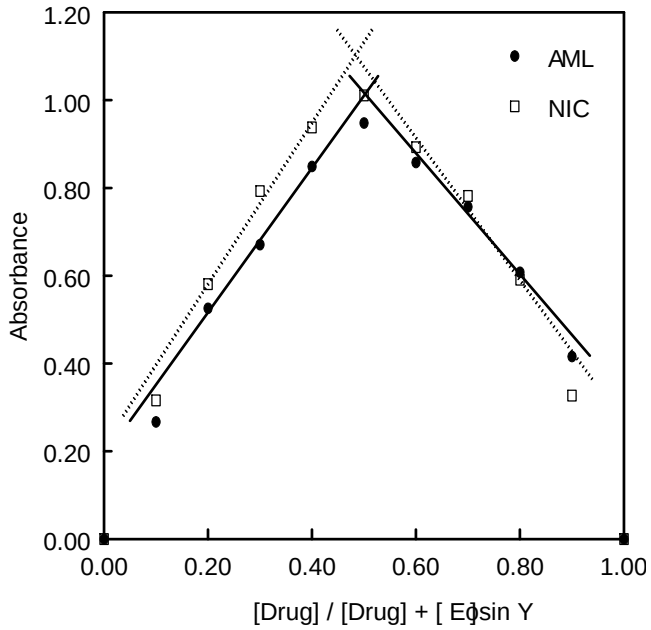

Fig. 5: Molar ratios obtained for the reaction of 1.5 x 10^{-3} M eosin Y with NIC (-□-) and AML (-●-) of the same molar concentration at room temperature.

Validation of the proposed methods

Under the specified optimum reaction conditions, the calibration curves for the reaction of the investigated drugs (NIC, and AML) with eosin Y were constructed. A good linear relationship was observed within the range 5.0-60.0 µg/ml. The effective molar absorptivity (e) was calculated from the slope of the calibration graph. The analytical data, e.g., Beer's law limit, the effective molar absorptivity (e), the regression equation (Y = a + bX, Y, absorbance; X, µg/ml; a, intercept; and b, slope), and the correlation coefficient (r) are summarized in Table 2.

Table. 2: Analytical parameters for the proposed method for determination of AML and NIC.

Parameter [a]	AML	NIC
Linear range (µg/ml)	5-60	10-60
Intercept (a) ± SD	0.0548 ± 0.0087	-0.0002 ± 0.005
Slope (b) ± SD	0.0144 ± 0.0003	0.0145 ± 0.0001
Correlation coefficient (r)	0.9991	0.9995
$\varepsilon \times 10^{-3}$(l mol^{-1} cm^{-1})	9471	7495
LOD (µg ml^{-1})	1.8	1.1
LOQ (µg ml^{-1})	6.0	3.6

* SD is Standard deviation

Accuracy and precision

The intra-day and inter-day precisions of the proposed method were examined by analyzing three (or five) replicates of the working standards at one concentration level for each drug. Relative standard deviations did not exceed 2% indicating the good reproducibility of the proposed method (Table 3).

Robustness

Robustness was examined by evaluating the influence of small variation of method variables such as eosin Y concentration and reaction time on the performance of the proposed method (Table 3). These variations do not have any significant affect on the recovery of the method.

Table 3: Influence of small variations in the assay conditions of eosin Y method on the suitability test parameters and sensitivity.

Parameters	% Recovery [a] ± SD	
	NIC (40 µg/ml**)**	**AML (**40 µg/ml**)**
No variation	100.06 ± 0.25	100.08 ± 1.23
Eosin Y concentration		
19.75 mg/ml	99.27 ± 0.95	100.13 ± 0.19
20.25 mg/ml	100.16 ± 0.15	100.44 ± 1.11
Reaction time		
8 min.	99.19 ± 0.21	99.58 ± 1.15
12 min.	100.09 ± 0.13	99.08 ± 0.19
Intra-day precision		
Day-1	99.17 ± 0.24	100.17 ± 0.26
Day-2	100.74 ± 1.22	100.27 ± 1.32
Day-3	100.81 ± 0.44	100.38 ± 0.98
Inter-day precision	100.55 ± 0.32	100.17 ± 0.23

[a] Average of three determinations except five determinations for inter-day precision.

Specificity and interference

Potential interference of the common tablet excipients were tested using starch, glucose, magnesium stearate and talc. Samples were prepared by mixing 20 mg of either NIC or AML with the recommended amounts [17] of the tablet excipients. The recovery value was 97.3-99.5 ± 0.14-1.39 %. This indicated the absence of interference from these excipients (Table 4). Moreover, the proposed method is performed at 549 nm in the visible region away from the UV-absorbing capabilities of interferences that might be co-extracted from laboratory prepared tablet.

Table. 4: Analysis of AML and NIC in the presence of commonly used pharmaceutical excipients.

Excipients (mg)	Amount of excipients added (mg)	Recovery (% ± SD) [a]	
		NIC	AML
Starch	50	98.20 ± 0.69	97.3 ± 0.28
Glucose	10	98.50 ± 1.22	97.6 ± 1.39
Mg stearate	5	99.1 ± 1.08	98.3 ± 0.14
Talc	5	99.5 ± 0.25	98.2 ± 0.36

[a] The values are average of five determinations ± SD.
[b] The amount of excipients added per 20 mg of NIC and AML.

Application of the proposed methods

The proposed method was applied for the analysis of studied drugs in their commercial pharmaceutical dosage forms. The drug concentration was computed from its corresponding regression equations. The results of the proposed method were statistically compared with those of the reported method(Huang and Li 1990; Basavaiah *et al.* 2003), in respect to the accuracy and precision. The obtained mean recovery values of the labeled amounts were 97.1 ± 0.69 - 99.8 ± 0.58 % (Table 5). The results obtained were in good agreement with those obtained using the reference method. Student's t-test and the variance ratio F-test revealed no significant differences between the performance of the two methods regarding the accuracy and precision, respectively.

Table 5: Determination of AML and NIC in their pharmaceutical dosage forms by the reported and the proposed methods.

Product	% Recovery [a] ± SD		F-value[b]	t-value[b]
	Proposed method	Reported methods[c]		
Pelcard SR® tablets.	97.6 ± 0.69	99.7 ± 0.11	2.00	1.11
Alkapress® tablets.	99.8 ± 0.58	99.2 ± 0.12	1.16	1.54
Myodura® tablets	98.3 ± 0.78	98.3 ± 0.11	1.45	2.74
Amlodipine® tablets	97.1 ± 0.69	97.2 ± 0.12	1.86	1.96
Regcor® tablets	97.3 ± 1.14	99.2 ± 0.16	2.73	0.67
Vasonorm® tablets	99.5± 0.70	98.4 ± 0.19	2.44	2.12

[a] Reference, values are mean ± RSD of five determinations. [b] The tabulated values of *t* and *F* at 95% confidence limit are 2.78 and 6.39, respectively. [c] Reference (Huang and Li 1990; Basavaiah *et al.* 2003).

CONCLUSION

The present study described fully validated new spectrophotometric methods for the determination of AML and NIC in their pharmaceutical formulations with enhanced selectivity. The proposed method does not require elaborate treatment of the samples and/or tedious procedures for extraction required with the chromatographic and other traditional extractive spectrophotometric methods. As well, the method is sensitive enough for analysis of low concentration of studied drugs. Furthermore, the proposed methods do not require expensive instruments and/or critical analytical reagents. These advantages give the proposed method a great value and make it applicable for the analysis of two drugs in quality control laboratories.

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