SPECTROPHOTOMETRIC DETERMINATION OF ATORVASTATIN AND AMLODIPINE BY H-POINT STANDARD ADDITION METHOD WITH SIMULTANEOUS ADDITION OF BOTH ANALYTES IN NONAQUEOUS SOLUTION

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Received December 13, 2010
Accepted January 26, 2011
Published online February 21, 2011

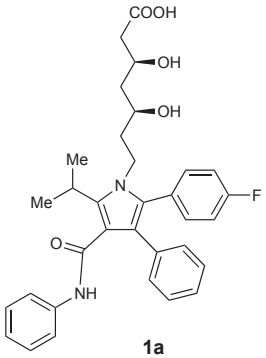
H-point standard addition method(HPSAM) with simultaneous addition of both analytes in nonaqueous solution was applied for determination of Atorvastatin (AT) and Amlodipine (AML). The results showed that simultaneous determination could be performed with the ratio 0.16 to 4.3 of AT–AML. The corresponding values of LOD were obtained for AT and AML 1.2×10^{-6} and 9×10^{-7} mol l^{-1}, respectively, and the corresponding values of LOQ were obtained for AT and AML 4×10^{-6} and 3×10^{-6} mol l^{-1}, respectively. Underworking conditions, the proposed method was successfully applied for simultaneous determination of AT and AML in several synthetic mixtures and Amostatine tablets.

Keywords: Analytical methods; Computational chemistry; Medicinal chemistry; H-Point standard addition method; Atorvastatin; Amlodipine; Amostatine; HPLC.

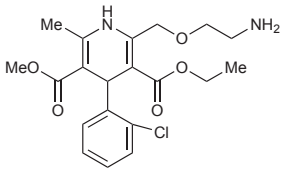
Atorvastatin (AT), (3*R*,5*R*)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic hemicalcium salt (Fig. 1a), is a synthetic lipid-lowering agent with very slightly solublility in distilled water. It is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, an enzyme that catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis[1]. This HMG-CoA reductase inhibitor can efficiently reduce both cholesterol (25 to 61%) and triglyceride (9 to 61%) levels in hyperlipidemic patients[2–4]. It has also been reported to reduce the levels of apolipoprotein B, and intermediate density lipoprotein[5]. Atorvastatin, in tablet form, is indicated as an adjunct to diet for prevention of cardiovascular diseases and for treatment of patients with hypercholesterolemia, primary dysbetalipoproteinemia, or other forms of dyslipidemia[6–8]. It is also used to reduce the

doi:10.1135/cccc2010144

risk of myocardial infarction, stroke, and heart failure in people with type 2 diabetes without evidence of heart disease[9,10]. Atorvastatin is absorbed rapidly following administration, reaching the plasma concentration peak within 4 h. Amlodipine *R*,*S*-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine (AML) with slightly solubility in water (Fig. 1b) is a potent, long-lasting calcium antagonist agent, widely used against hypertension and angina[11,12]. Amlodipine belongs to the third generation antagonists which retain tissue selectivity and show a favourable pharmacokinetic profile and in combination with other drugs, it is one of the most widely prescribed cardiovascular drugs[13]. Recently the combination of AT and AML is used for fibrinolysis in hypertensive hypercholesterolemic patients with insulin resistance[14]. Also van de Poll and coworkers, quantified the anti-atherosclerotic potential of Amlodipine, Atorvastatin and their combination on existing atherosclerotic plaques in the aorta of APOE-3-Leiden transgenic mice[15]. Leibovitz and coworkers demonstrated that AML and AT have an additive effect in improvement of arterial compliance in hypertensive hyperlipidemic patients[16]. Amostatine (trade name used in Iran) is a fixed combination of AT and AML widely used for treatment of cardiovascular diseases. According to literature survey, there have been several reports of determination of AT or AML in biological samples. The most popular analytical method for this active pharmaceutical ingredient (API) quantification for AT and AML is UV-Vis spectrophotometry[17,18], high-performance liquid chromatography equipped with mass spectrometry (HPLC-MS)[19–22] or UV (HPLC-UV) detector[23–25] and also electrophoresis[26]. The chromatographic methods are sensi-



1a



1b

FIG. 1
The structure of Atorvastatin **1a** and Amlodipine **1b**

tive and specific, but these devices are expensive and need highly trained persons. The suitability of UV-Vis spectrophotometric method for quantitative analysis of AT and/or AML in pharmaceutical samples is well documented[27,28]. The most important advantages of UV-Vis technique are simplicity, short analysis time, its capacity for automation and economic advantages. Although the UV-Vis method offers desired advantages but it suffers from some practical difficulties. The advantages of this technique is often overshadowed by interference of AT peaks with AML spectrum. Therefore, accurate determination of these two drugs in presences of each other is not possible by a common manner. Dinc and Baleanu used the principal component regression (PCR) and partial least squares (PLS) chemometric methods for simultaneous quantitative analysis of AT and AML in tablets without using a preliminary separation and in presence of the overlapping spectra of drugs[29]. Although these methods have advantages for full peak analysis and adding more latent variables to calibration data could be applied for solving problems of non linearity. However, deep understanding of these methods is not easy for some users. Chaudari and Patel proposed a dual wavelength spectrophotometric method for simultaneous determination of AT and AML in tablets[30]. The choice of wavelengths was performed form the pure spectrum of AML and AT in a pure solvent, while in real samples each tablet has specific excipients. Therefore, failure to subtract of signal of blank or TYB from the total signal produces biased results. The H-point standard addition method (HPSAM) has been introduced by Campins-Falco and et al.[31] as a simple two variable chemometric technique for determination of the concentration of the analyte that is free from both proportional and constant errors in the presence of a known interferent. Also, in 1995 Campins-Falco and coworkers[32] proposed a modified HPSAM with simultaneous addition of both analytes, which permitted the resolution of both species from a unique calibration, set by simultaneous addition of both analytes. The HPSAM was firstly developed for spectrophotometric determination of two analytes which are soluble in aqueous solutions, while it is also capable of developing for determination of two lipophilic analytes in nonaqueous solutions[33]. In this work, the H-point standard addition method with simultaneous addition of AT and AML has been applied for analyzing of binary mixtures of AT and AML in methanol using the UV-Vis spectrophotometric method and comparison of results with the results of HPLC analysis.

EXPERIMENTAL

Apparatus

A detection system consists of a Shimadzu (UV 2450) UV-Vis spectrophotometer, equipped with a 1-cm quartz cell and a temperature controller (CPS 240A). The spectrophotometer was interfaced to a personal computer and furnished with UV-probe software (ver. 2.2). The chromatographic analysis was carried out by a KNAURE instrument equipped with a power supplier and a UV detector. The HPLC was controlled by EZ-Chrome Elite software. The separation was performed on a Eurospher 100-5C18 column (240 × 4.0 mm i.d.). The mobile phase, consisted of MeOH–acetonitrile–0.05 M phosphate buffer (pH 3.0, 40:35:25 v/v%). The column was placed at an oven at 40 °C. The flow rate was 1.0 ml min^{-1}, and the detection wavelength was 237 nm. The injection volume was 20 µl and the run time was 7.0 min.

Reagents

Atorvastatin and Amlodipine were purchased from Sigma (St. Louis, USA). All other reagents and solvents were purchased from Merck. A stock solution of AT (1×10^{-3} mol l^{-1}) and AML (1.2×10^{-3} mol l^{-1}) were prepared by dissolving 0.0056 g of AT and 0.0049 g of AML in MeOH and diluting to the mark with MeOH in a 10.0 ml volumetric flask. Amostatine tablets of two different batches were purchased from a local pharmacy store.

RESULTS AND DISCUSSIONS

Theoretical Background

Due to the low solubility of AT and AML in water, the MeOH was used as a suitable medium for development of HPSAM with simultaneous addition of two analytes. In our laboratory, the same theory was used for simultaneous adsorptive stripping voltammetric determination of cadmium and uranium by using LDOPA as a complexing agent[34]. A typical spectrum of a binary mixture of AT and AML is shown in Fig. 2.

As it is observed, the absorbance of AML + AT in each wavelength is the sum of individual absorbances of AML and AT

$$A_i = K_{i,\mathrm{AT}} C^0_{\mathrm{AT}} + K_{i,\mathrm{AML}} C^0_{\mathrm{AML}} + A^0_i \qquad (1)$$

where A_i is the absorbance of the mixture at wavelength λ_i, C^0_{AT} and C^0_{AML} are concentrations of AT and AML in MeOH solution, $K_{i,\mathrm{AT}}$ and $K_{i,\mathrm{AML}}$ are proportional coefficients at λ_i for corresponding drugs and A^0_i is the residual absorbance at λ_i, which can be nearly omitted by subtracting the absorbance of blank. So, Eq. (*1*) can be written as

$$A_i = A^0_{i,\mathrm{AT}} + A^0_{i,\mathrm{AML}} + A^0_i \qquad (2)$$

where $A^0_{i,AT}$ and $A^0_{i,AML}$ are the individual absorbances of the AT and AML drugs in the considered sample. According to HPSAM basis, the quantification of each analyte X (AT or AML) in the presence of the other interferent Y, can be performed by the construction of two standard addition plots for the analyte, with $M_{X,1}$ and $M_{X,2}$ slopes, at two previously selected wavelengths, λ_1 and λ_2, which intersect at the H-point with ($C_{H(X)}$, $A_{H(Y)}$) coordinates. For example if AT is considered as the analyte, the H-point depends on its concentration C^0_{AT}.

$$-C_{H(AT)} = \frac{\left(A^0_{AT,1} - A^0_{AT,2}\right) + \left(A^0_{AML,1} - A^0_{AML,2}\right)}{M_{AT,2} - M_{AT,1}} = -C^0_{AT,1}\,\frac{A^0_{AML,1} - A^0_{AML,2}}{M_{AT,2} - M_{AT,1}} \qquad (3)$$

By selecting λ_1 and λ_2 in a way that the AML absorbance values are equal $A_{AML,1} = A_{AML,2}$ then the abscissa of the H-point will be the AT concentration in the sample, C^0_{AT}. The concentration of AML drug can be determined according to conventional HPSAM from the AH value and a calibration plot obtained separately for AML drug. HPSAM based on simultaneous standard addition of the two species as reported by Falco et al.[32] permits one to obtain concentration of both analytes in the sample from a unique calibration set. The required data to apply the method are the response of the sample and the response of the sample spiked with known amounts of both analytes at previously selected wavelengths. For simultaneous determination of AT and AML by spectrophotometric method, let us suppose that λ_1 and λ_2 are selected according to Eq. (*3*). Because the addition is made from a standard mixture of both analytes, the relation of the added concen-

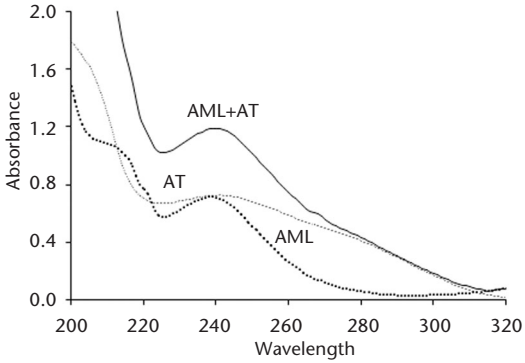


FIG. 2
The spectrum of 1.0×10^{-5} M AT, 1.0×10^{-5} M AML and AML+AT in the MeOH solution

trations between species AT and AML is the same in all of the solutions prepared to apply the method. By representing the analytical signal, absorbance at two previously selected wavelengths λ_1 and λ_2 versus the added concentration of AT, two lines would be obtained with intercepts $(A^0_{AT,1} + A^0_{AML,1})$ and $(A^0_{AT,2} + A^0_{AML,2})$ and slopes

$$\text{slope at } \lambda_1\text{: } M_{AT,1} + \left(\frac{C^i_{AML}}{C^i_{AT}}\right) M_{AML,1} \qquad i = 0,\ 1,\ \ldots,\ n$$

$$\text{slope at } \lambda_2\text{: } M_{AT,2} + \left(\frac{C^i_{AML}}{C^i_{AT}}\right) M_{AML,2} \qquad i = 0,\ 1,\ \ldots,\ n$$

$M_{AT,1}$, $M_{AML,1}$, $M_{AT,2}$ and $M_{AML,2}$ are the slopes due to the addition of AT and AML in the lines obtained at λ_1 and λ_2, C^i_{AT} and C^i_{AML} are the concentrations of considered drugs added in the i solution, n is the number of additions. $i = 0$ corresponds to the solution when only the sample exists. Both calibration lines intersect at the H-point, with coordinates $(-C_{H(AT)},\ A_{H(AML)})$, where $-C_{H(AT)}$ is the unbiased concentration of AT. In this case the abscissa of the H-point will be

$$-C_{H(AT)} = \frac{\left(A^0_{AT,1} - A^0_{AT,2}\right) + \left(A^0_{AML,1} - A^0_{AML,2}\right)}{(M_{AT,2} - M_{AT,1}) + \left(\frac{C^i_{AML}}{C^i_{AT}}\right)(M_{AML,2} - M_{AML,1})} =$$

$$= \frac{\left(A^0_{AT,1} - A^0_{AT,2}\right)}{(M_{AT,2} - M_{AT,1})}\,. \qquad (4)$$

The incorrigible error due to the presence of AML, in spite of its concentration is not constant, has been transformed into a constant systematic error as the HPSAM basis predicts. It can be proved that $A_{H(AML)}$ is equivalent to

$$A_{H(AML)} = A^0_{AML,1} - \left(\frac{C^i_{AML}}{C^i_{AT}}\right) M_{AML,1}\, C_{H(AT)} =$$

$$= A^0_{AML,2} - \left(\frac{C^i_{AML}}{C^i_{AT}}\right) M_{AML,2}\, C_{H(AT)}\,. \qquad (5)$$

In the same way as for AT, by selecting two potentials λ_3 and λ_4 in such a way that the AT presents the same absorbance $A^0_{AT,3} = A^0_{AT,4}$ similar expressions can be obtained for AML. The analogous expressions for Eqs (*4*) and (*5*) in analysis of AML are

$$-C_{H(AML)} = \frac{\left(A^0_{AML,3} - A^0_{AML,4}\right) + \left(A^0_{AT,3} - A^0_{AT,4}\right)}{(M_{AML,4} - M_{AML,3}) + \left(\frac{C^i_{AT}}{C^i_{AML}}\right)(M_{AT,4} - M_{AT,3})} =$$

$$= \frac{\left(A^0_{AML,3} - A^0_{AML,4}\right)}{(M_{AML,4} - M_{AML,3})} \qquad (6)$$

and

$$A_{H(AT)} = A^0_{AT,1} - \left(\frac{C^i_{AML}}{C^i_{AT}}\right) M_{AT,3}\, C_{H(AML)} =$$

$$= A^0_{AT,3} - \left(\frac{C^i_{AML}}{C^i_{AT}}\right) M_{AT,4}\, C_{H(AML)} \,. \qquad (7)$$

For selection of appropriate wavelengths of λ_1, λ_2, λ_3 and λ_4 for application HPSAM with simultaneous addition of both analytes, as mentioned above, the following principles must be considered. At two selected wavelengths (λ_1 and λ_2 or λ_3 and λ_4) the analyte signals (AT or AML) must be linear with the concentration, the interferent signals or second analyte (AML or AT) must remain equal, and the analytical signals of the mixture composed of both compounds should be equal to the sum of the individual signals of two compounds. In addition, the slope difference of the two straight lines obtained at λ_1 and λ_2 or λ_3 and λ_4 must be as large as possible in order to get good accuracy and sensitivity. As it can be seen from the spectra (Fig. 2), there are several pairs of wavelengths that fulfill the requirement for application of HPSAM. The best results in terms of linearity (correlation coefficients greater than 0.99), precision and accuracy are generally obtained for the wavelengths pairs of 223 and 251 nm for determination of AT and 284 and 306 nm for determination of AML. In addition, these two pair's wavelengths caused the largest difference between the slopes of standard addition lines $M_{AT,\lambda 2} - M_{AT,\lambda 1}$ and $M_{AML,\lambda 2} - M_{AML,\lambda 1}$. At H-point, CH is

independent of the concentration of interferent and AH is also independent of the analyte concentration. Figures 3a and 3b clearly show the effects of concentration of AT and AML on the position of the H-point. However, as shown in Figs 3a and 3b the value of CH for each analyte (X: AT or AML) is independent of the concentration of interferent (Y: AML or AT) in the sample. Four synthetic mixtures with different concentrations of AT and AML were prepared in MeOH and were analysed using the suggested

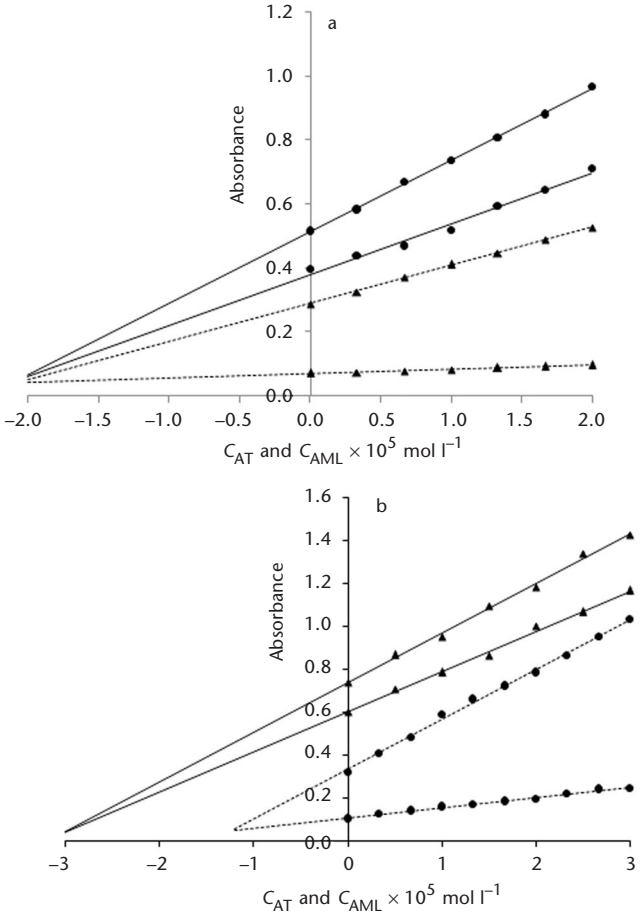


FIG. 3
Plots of HPSAM for simultaneous determination of (a) 2.0×10^{-5} M AT (▲) and 2.0×10^{-5} M AML (●) and (b) 3.0×10^{-5} M of AT (▲) and 1.2×10^{-5} M of AML (●)

method. The results are given in Table I. As it can be seen from Table I, the accuracy of the analysis is satisfactory when the concentration ratio of AT/AML varies from 0.16 to 4.3.

Reproducibility of the Method

Under optimum conditions, simultaneous determination of AT and AML were made by using proposed HPSAM. To check the reproducibility of the method, four replicate experiments of AT and AML determination were car-

TABLE I
Results of four experiments for the analysis of AT–AML mixtures in different concentration ratios

A–C equation	R^2	Sample ($\times 10^{-5}$ M)		Found ($\times 10^{-5}$ M)	
		AML	AT	AML	AT
$A_{284} = 0.269C + 0.325$	0.995	1.2	3	1.16 ± 0.02	2.96 ± 0.04
$A_{306} = 0.071C + 0.096$	0.999				
$A_{223} = 0.200C + 0.682$	0.997				
$A_{251} = 0.149C + 0.531$	0.998				
$A_{284} = 0.445C + 0.310$	0.999	0.7	3	0.68 ± 0.01	3.05 ± 0.05
$A_{306} = 0.122C + 0.091$	0.997				
$A_{223} = 0.212C + 0.671$	0.998				
$A_{251} = 0.189C + 0.601$	0.999				
$A_{284} = 0.027C + 0.115$	0.999	3.6	1	3.66 ± 0.04	1.03 ± 0.02
$A_{306} = 0.009C + 0.049$	0.996				
$A_{223} = 0.389C + 0.569$	0.999				
$A_{251} = 0.328C + 0.506$	0.997				
$A_{284} = 0.021C + 0.091$	0.998	3.6	0.6	3.64 ± 0.04	0.64 ± 0.01
$A_{306} = 0.007C + 0.040$	0.994				
$A_{223} = 0.502C + 0.451$	0.999				
$A_{251} = 0.425C + 0.402$	0.996				

ried out (Table II). A good RSD (%) was obtained for AT and AML as is given in Table II. The limit of detection (LOD) and limit of quantification (LOQ) were measured as LOD = 3 Sc and LOQ = 10 Sc, respectively, where Sc is the standard deviation of several (n = 4) replicated measurements of zero concentration of each analyte with HPSAM [35,36]. The corresponding values of LOD obtained for AT and AML were 1.2×10^{-6} and 9×10^{-7} mol l^{-1}, respectively, and the corresponding values of LOQ obtained for AT and AML were 4×10^{-6} and 3×10^{-6} mol l^{-1}, respectively.

TABLE II
Results of four replicate experiments for the analyses of AT–AML mixtures

A–C equation	R^2	Sample ($\times 10^{-5}$ M)		Found ($\times 10^{-5}$ M)	
		AML	AT	AML	AT
$A_{284} = 0.065C + 0.144$	0.998	2	2	2.04	2.03
$A_{306} = 0.019C + 0.051$	0.999				
$A_{223} = 0.237C + 0.541$	0.997				
$A_{251} = 0.208C + 0.482$	0.998				
$A_{284} = 0.063C + 0.146$	0.998	2	2	2.02	2.0
$A_{306} = 0.018C + 0.055$	0.998				
$A_{223} = 0.235C + 0.544$	0.999				
$A_{251} = 0.207C + 0.488$	0.996				
$A_{284} = 0.067C + 0.146$	0.997	2	2	1.98	2.07
$A_{306} = 0.020C + 0.053$	0.997				
$A_{223} = 0.237C + 0.551$	0.998				
$A_{251} = 0.210C + 0.495$	0.996				
$A_{284} = 0.065C + 0.140$	0.998	2	2	2.04	1.97
$A_{306} = 0.020C + 0.048$	0.998				
$A_{223} = 0.231C + 0.548$	0.999				
$A_{251} = 0.203C + 0.493$	0.997				
Mean				2.02	2.02
Standard deviation				0.028	0.0428
RSD, %				1.4	2.1

TABLE III
AT and AML determination in several Amostatine samples using the proposed methodology and HPLC

Amostatin samples	Found AML mg/tablet proposed method	Found AT mg/tablet proposed method	Found AML mg/tablet HPLC	Found AT mg/tablet HPLC
1	5.06	20.37	5.09	20.21
2	5.01	19.48	5.10	19.88
3	5.17	20.22	4.93	20.16
4	5.05	19.92	5.02	20.69
5	5.11	19.77	4.91	20.08
6	4.96	20.07	5.06	20.35
Mean	5.06	19.97	5.02	20.22
Standard deviation	0.074	0.321	0.08	0.274
RSD, %	1.46	1.61	1.59	1.36

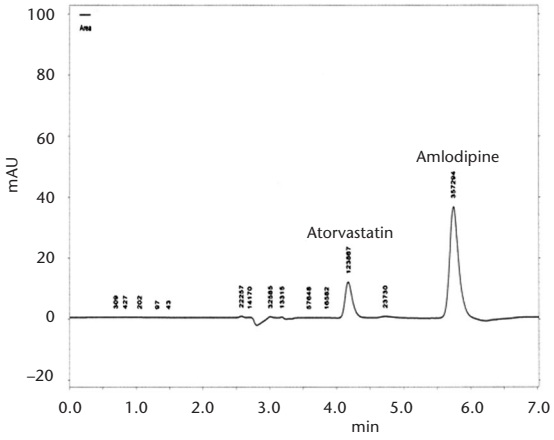


FIG. 4
A sample chromatogram of Atorvastatin and Amlodipine tablets: the mobile phase contained MeOH–acetonitrile–0.05 M phosphate buffer (pH 3.0, 40:35:25 v/v%), the wavelength of detection was 237 nm

Analytical Application

The applicability of the developed method was further assessed through determining the AT and AML in several Amostatine tablets. Samples were prepared according to our pervious works[37,38]. AT and AML were extracted from amostatine tablets using the MeOH as an appropriate solvent and analysed using the proposed method. The samples were also analysed by HPLC (Fig. 4) and the results were compared (Table III). As it is observed from Table III, these results indicated that there are no significant differences between the outputs of the HPLC and the proposed method in nonaqueous solution. No significant differences were observed in AT and AML contents in each tablet (t-test at $p = 0.05$) between the HPSAM results and the contents labeled by the manufacturer.

CONCLUSION

The present study demonstrated that AT and AML drugs can be determined by using H-point standard addition method with simultaneous addition of both analytes in nonaqueous solution. This method offers a practical potential for simultaneous determination of AT and AML in Amostatine tablets with an acceptable selectivity, sensitivity, simplicity and speed.

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