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ECOFRIENDLY VALIDATED SPECTROPHOTOMETRIC METHODS FOR THE ESTIMATION OF AMLODIPINE BESYLATE IN PURE AND PHARMACEUTICAL FORMULATIONS USING HYDROTROPIC SOLUBILIZATION

Makka Hashim Ahmed*[1], Mohamed El-Mukhtar[1] and A. E. M. Saeed[1]

[1]Department of Chemistry, College of Science, Sudan University of Science and Technology.

***Corresponding Author: Makka Hashim Ahmed**

Department of Chemistry, College of Science, Sudan University of Science and Technology.

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ABSTRACT

Simple sensitive and accurate spectrophotometric methods were developed for determination of poorly water soluble Amlodipine Besylate (AML) in pure and pharmaceutical dosage form using three hydrotropic agents ammonium acetate (method 1), potassium acetate (method 2) and nicotineamide (method 3). Hydrotropy is a good choice for replacing organic solvents used for this kind of drugs to reduce the cost and hazards of the analytical method. Amlodipine Besylate shows maximum absorption at 365 nm in 0.5 M of the three hydrotropic agents. All hydrotropic agents did not show any absorbance above 250 nm so there is no interferences. Beer's law was found to be obeyed in the concentration range of 5 - 90 µg/mL for the three hydrotropes. Limit of detection was found to be 1.072, 0.646 and 0.866 µg/mL and limit of quantification was 3.249, 1.956 and 2.625 µg/mL for ammonium acetate, potassium acetate and nicotineamide, respectively. The results were in a good agreement with those obtained with official USP method.

KEYWORDS: Amlodipine Besylate, colorimetry, hydrotropy, method validation.

1 INTRODUCTION

Amlodipine besylate is a long-acting calcium channel blocker. It is chemically described as 3-ethyl-5-methyl(±)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, monobenzenesulphonate. Its empirical formula is $C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$ and its structural formula as in figure. 1.[1] It is used alone or in combination with other medications to treat high blood pressure and chest pain (angina).[2] Amlodipine besylate inhibits the trans-membrane influx of calcium ions into vascular smooth muscles and cardiac muscle. It is a peripheral arterial vasodilator that acts directly on vascular smooth muscles to cause a reduction in peripheral vascular resistance and reduction in blood pressure. It is effective in both types of angina exertional and vasoartic.[3]

Several methods were reported for the estimation of amlodipine besylate such as HPLC[4-7], HPTLC[8-10], LC-Ms/Ms[11,12], spectrofluorometry[13] and spectrophotometry.[14-19]

Three methods were reported for quantification of amlodipine besylate using hydrotropic agent. These methods include utilization of 1 M sodium acetate, 2 M sodium acetate and 2 M urea.[20-22] These methods suffer from low sensitivity due to high values of limit of detection and limit of quantification and also utilize concentrated solution of hydrotropic agents.

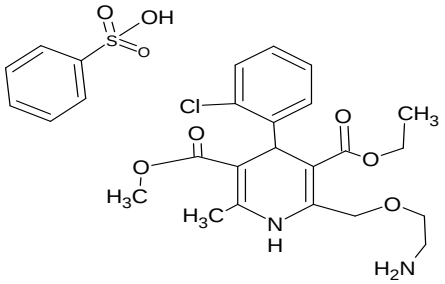

Fig.1 Chemical structure of amlodipine besylate

2 MATERIAL AND METHODS

2.1 Materials and chemicals

Amlodipine besylate with 99.66% purity was obtained from Amipharma Laboratories Ltd, Khartoum, Sudan as a gift. Ammonium acetate, potassium acetate and nicotineamide were purchased from Aldrich Chemical Co., St. Louis, USA. Amlodipine besylate tablets containing 10 mg AML, Amipharma, Sudanese product were obtained from a local market of Sudan.

2.2 Instruments

UV spectra of samples were recorded on Shimadzu 1800 double beam spectrophotometer using quartz cell of 10 mm path length and UV Probe software. KAREN electrical analytical balance (0.1 mg – 120 g) is used for weighing the samples.

2.3 Preparation of standards, sample and reagents solutions

2.3.1 Preparation of ammonium acetate, potassium acetate and nicotineamide solutions

0.5 M solution of ammonium acetate, potassium acetate and nicotineamide were prepared by dissolving 3.853, 4.908 and 6.106 g of ammonium acetate, potassium acetate and nicotineamide, respectively in distill water and makeup the volume to 100 mL with distill water.

2.3.2 Preparation of drug solution

A stock solution of (200 µg/ml) was prepared by dissolving 20 mg of AML standard in 50 mL of 0.5M hydrotropic agent, sonicated for 20 minutes, then makeup to 100 mL with distill water.

2.3.3 Preparation of sample solution

20 tablets of amlodipine besylate (10 mg/tablet) were weighed and finely powdered. A portion of the powder equivalent to 10 mg of drug was dissolved in 50 mL of hydrotropic agent solution, sonicated for 20 minutes, filtered and makeup to 100 mL with distill water to give a solution of 100 µg/mL.

2.4 Procedure for calibration curve

Serial concentrations were prepared from the stock solution by taking 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 and 4.5 mL to 10 mL calibrated flask and the volume was makeup to 10 mL with distill water. Absorbance of these solutions was recorded at λ_{max} 365 nm against blank solution treated in the same way omitting only the drug.

2.5 Application of the proposed method to analysis of Amlodipine besylate dosage form

Amlodipine besylate tablets were subjected to the analysis by the proposed methods as well as with the official HPLC method (United States Pharmacopeia) and the obtained results were statistically analyzed.

3 RESULTS AND DISCUSSION

3.1 Absorption spectra

According to the procedure, the absorption spectrum of AML in ammonium acetate, potassium acetate and nicotineamide was recorded in "Fig. 2". As shown in "Fig. 2", the maximum absorption wavelength (λ_{max}) was at 365 nm for the three hydrotropic agents.

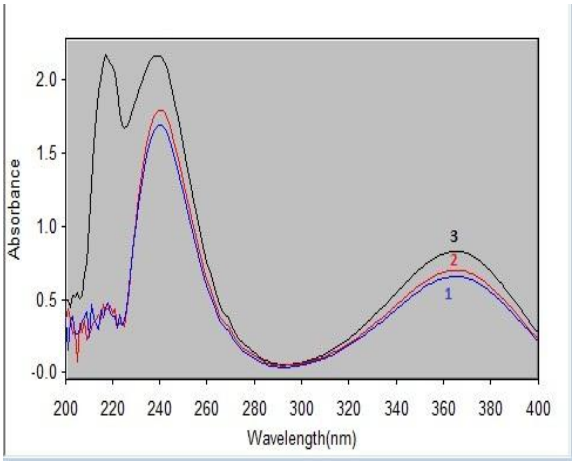

Fig. 2: Absorption spectra of AML in (1) potassium acetate, (2) ammonium acetate and (3) nicotinamide.

3.2 Method validation

3.2.1 Linearity, range, limit of detection and limit of quantification

The linearity of the methods was investigated and found to be in the range of 5- 90 µg/mL for the three methods as shown in "Fig. 3". Regression equation, Beer's law limits, slope, intercept, correlation coefficient, Sandell's sensitivity, molar absorptivity, limit of detection (LOD) and limit of quantification (LOQ) were summarized in Table. 1.

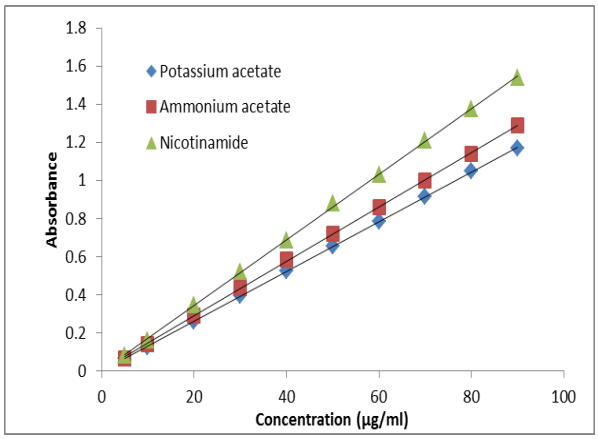

Fig. 3: Calibration curves for determination of AML using hydrotropic agents

Table 1: Analytical Parameters for the determination of AML using hydrotropic agents.

Parameter	Value		
	Method 1	Method 2	Method 3
λ_{max} (nm)	365	365	365
Beer's law limits (µgmL^{-1})	5 - 90	5 - 90	5-90
Sandell's sensitivity (µgcm^{-2})	0.0581	0.0767	0.0699
Molar absorptivity (Lmol^{-1}.cm^{-1})	0.977×10^4	0.740×10^4	0.812×10^4
Std. Dev. of intercept	0.005598	0.002551	0.003759
LOD (µgmL^{-1})	1.072	0.646	0.866
LOQ (µgmL^{-1})	3.249	1.956	2.625
Slope (m)	0.0172	0.01304	0.0143
Intercept (b)	- 0.000295	0.000735	0.0084
Correlation coefficient	0.9997	0.9999	0.9998

3.2.2 Precision

To assess the precision, each experiment was repeated three times on the same day (intra-day) and on different days (inter-day). The results show the methods are precise according to the law values of standard deviation (SD) and percent relative standard deviation (% RSD), as shown in Table. 2.

Table 2: Evaluation of intra- and inter-day precision.

Method	Taken (μgmL^{-1})	Intra – day			Inter – day		
		Found (μgmL^{-1})	SD	% RSD	Found (μgmL^{-1})	SD	% RSD
Method 1	10	10.098	0.058	0.476	10.190	0.049	0.476
	20	20.179	0.034	0.166	20.217	0.051	0.253
	30	30.008	0.058	0.193	30.050	0.039	0.130
Method 2	10	9.975	0.044	0.444	9.941	0.039	0.393
	20	20.047	0.044	0.221	20.029	0.053	0.266
	30	30.272	0.077	0.253	30.186	0.078	0.259
Method 3	10	9.988	0.0404	0.404	9.957	0.036	0.358
	20	20.104	0.040	0.201	20.088	0.0931	0.464
	30	30.361	0.001	0.464	30.322	0.036	0.117

3.2.3 Accuracy & Recovery

Accuracy is estimated in terms of percent recovery and percent relative standard deviation. t-test and F-test values have been calculated using USP standard reference HPLC method. The t-test and F-test values are less than their permissible values (2.776 and 19.0 for t and F, respectively at 95% confidence level), indicating a high accuracy and precision of the methods as shown in Table. 3.

Table 3: Application of proposed methods for the analysis of the studied drug in pharmaceutical formulations.

Method	Taken (μgmL^{-1})	Found (μgmL^{-1})	% Recovery	% RSD	Proposed Method Mean ± SD	Reference Method Mean ± SD	t-test	F-test
Method 1	5	5.009	100.172	0.263	100.04 ± 0.26	99.77 ± 0.30	1.155	1.316
	10	10.020	100.201					
	20	19.946	99.732					
Method 2	5	5.016	99.558	0.263	99.74 ± 0.26		0.114	1.319
	10	9.975	99.626					
	20	20.046	100.043					
Method 3	5	4.9995	99.991	0.161	99.83 ± 0.16		0.315	4.317
	10	9.965	99.879					
	20	19.988	99.939					

3.24.4 Robustness

Robustness was examined by evaluating the influence of small variation in the method variables on its analytical performance. In these experiments, one parameter was changed whereas the others were kept unchanged and the recovery percentage was calculated each time. It was found that small variation in the method variables did not significantly affect the procedure's recovery values, as shown in Table. 4.

Table 4: Robustness of the proposed spectrophotometric methods.

Recommended condition		Recovery (Mean ± SD)*		
		Method 1	Method 2	Method 3
Standard		100.0351 ± 0.263	99.742 ± 0.2624	101.1404 ± 0.217
λ_{max}	363	100.008 ± 0.341	99.641 ± 0.444	100.275 ± 0.106
	367	99.814 ± 0.582	100.017 ± 0.221	100.228 ± 0.200
Concentration of hydrotropic agent	0.4 M	99.911 ± 0.444	99.973 ± 0.277	99.972 ± 0.241
	0.6 M	100.105 ± 0.290	100.024 ± 0.221	100.019 ± 0.223

***** Values are mean of three determinations.

4 CONCLUSION

The proposed methods were based on utilization of hydrotropic solubilization technique in developing Ecofriendly, simple and sensitive methods for quantitative estimation of amlodipine besylate in bulk and pharmaceutical dosage form. Method 2 is most sensitive due to the lowest values of LOD and LOQ as shown in Table 1, while method 3 is the most precise due to the lowest values of %RSD when applied in pharmaceutical dosage form as shown in Table 3. These methods can be applied for routine analysis in quality

control laboratories for the quantification of AML in both pure and pharmaceutical dosage forms.

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