International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.8, No.11 pp 229-236, 2015

A Spectrophotometric determination of Amlodipine Besylate (AMB) in Pharmaceutical Preparations using Gresol Red (GR) Reagent

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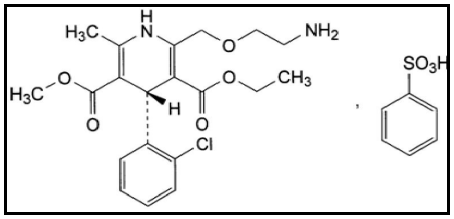
Abstract: A simple, rapid and sensitive spectrophotometric method for the determination of amlodipine besylate (AMB) in pure form and in pharmaceutical preparations has been developed. The proposed method is based on the formation of colored chloroform extractable ion-pair equal-molecular complexes of (AMB) with gresol red (GR) in britton pH 2 buffer. The extracted complexes showed absorbance maxima at 420nm. Beer´s law was obeyed in the concentration ranges(1.1-5.5µg.ml^{-1}). The molar absorptivities was 4.4110.10^{4} L.mol^{-1}.cm^{-1} and the Sandell`s sensitivity was 0.0257 µg.cm^{-2}, which indicated the high sensitivity of the proposed methods. The percent relative standard deviation 1.83% refered to the high accuracy and precision of the proposed method.

Key words: amlodipine besylate, gresol red, ion-pair complexes, spectrophotometric analysis, pharmaceutical preparations.

Introduction

Arnlodipine besylate (AMB) is a calcium channel chemicals blocking agent with vasodilatory activity similar to that used of nifedipine. It is mainly used for its antiarrhythmic, antianginal and antihypertensive activity (Heynen,1992)[1]. It is chemically known as 2-[(2- aminoethoxy)methyl]-4-(2-chloroprienyl)-1,4-dihydro-6-methyl-3,5-pyridine- dicarboxylicacid,3-ethyl,5 methylesterbesylate (Fig.1) [2]. Several analytical methods have been developed for the determination of AMB in their bulk, pharmaceutical formulations and biological fluids. Among the reported analytical methods for AMB are spectrophotometric methods[3-7], high performance liquid chromatography using fluorescence detection [8], UV detection [9-16] and LC-MS/MS [17-18]. In this work, a simple, sensitive, and accurate extractive spectrophotometric method was described for the determination of AML. This method is based on ion-pair complex formation between the drug (AMB) and gresol red (GR) in britton pH 2 buffer.



567.1 g. mol^{-1} : $C_{20}H_{25}ClN_2O_5$, $C_6H_6O_3S$ 2-[(2- aminoethoxy)methyl]-4-(2-chloroprienyl)-1,4-dihydro- 6-methyl-3,5-pyridine- dicarboxylicacid,3-ethyl,5 methylesterbesylate

Fig 1. The structure of amlodipine besylate (AMB)

Materials and methods

Instrumentation

All the absorption spectral measurements were made using a Optizen-3220 UV–Vis spectrophotometer with Wavelength Range 200–1100 nm and Spectral Bandwidth of 2 nm equipped with quartz cells.

Chemicals and reagents

- AMB tablets (contains amlodipine besylate equal to 5 mg amlodipine) were obtained from (Bahri Labs; Syria).
- Pure AMB ($C_{20}H_{25}ClN_2O_5$, $C_6H_6O_3S$ = 567.1 g. mol^{-1}) was obtained from Sigma-Aldrich (India). Its purity was found to be 99.68% according to (BP&USP Pharmacopia).
- Analytical-reagent gresol red (GR) was obtained from Merck.
- All used chemicals were purchased from Merck.

Standard solutions

- Stock solution of amlodipine besylate: 10 mg of AMB was weighed accurately into a 100 ml Volumetric flask. 50 ml of methanol were added and the mixture was shaken until AMB was completely dissolved. Then the solution was diluted to 100 ml with distilled water (100µg/ml) [2].
- Reagent solution: 382.4 mg of GR was dissolved in (ethanol:water;1:1) and diluted to 1000ml with distilled water (10^{-3}M) [19-20].
- Drug solution: Twenty tablets were weighed and pulverized to a fine powder. An aliquot equivalent to about 5 mg of AMB was transferred into a 100-ml volumetric flask. A suspension of the drug with 15 mL methanol and 25 ml water was shaken for 10 min and filtered to a second 100-ml volumetric flask. The first flask was rinsed with 3x10 ml water, which was transferred through the same filter paper. Final solution was diluted to 100 ml with distilled water (50µg/ml) [2].

Procedures

0.1-1.0 ml of standard solution of AMD was transferred into a series of 10 ml volumetric flasks. 3.5 ml of GR(1.10^{-3}M) solution was added to them, then 2ml of Britton buffer solution (pH =2) was added to them and the solutions were diluted to volume with distilled water. The reaction mixtures were extracted with (3ml) of chloroform for 2min using a vortex mixer, and allowed to stand for 5 min for separation of the chloroform layer. The absorbance of the chloroform phase was measured after an equilibrium time of 10 min in 1-cm quartz cells at 420 nm against blank solution, which was prepared similarly as the complexes except addition of the drug substances. The standard calibration plot was prepared to calculate the amount of the analyst drug in unknown samples. The colour is stable for at least 24 hrs up to 30 °C.

Results and discussion

Spectral characteristics

Containing cationic nitrogen, the cited drug reacts with GR to form a yellow ion-pair complex between the basic nitrogen of the drug in Britton buffer and GR Each drug-dye complex, with two oppositely charged ions, behaves as a single unit held together by an electrostatic force of attraction [21-23]. The complex is quantitatively extracted into chloroform. Absorption spectrum of the yellow AMB-GR ion-pair complex extracted into chloroform with its λmax at 420 nm is shown in Fig.2. The colorless blank has practically negligible absorbance.

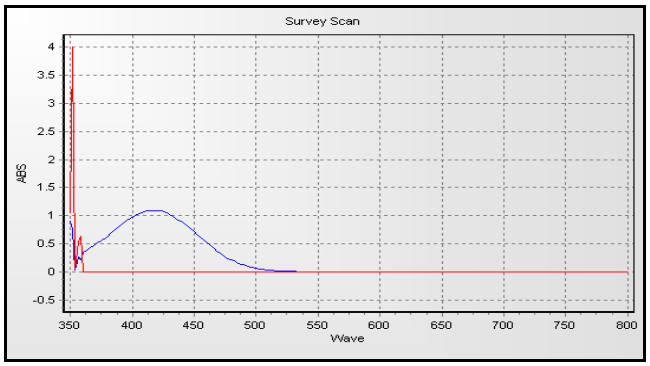


Fig 2. Absorption spectra of AMB-GR complex

Optimization of variables

Optimum conditions necessary for rapid and quantitative formation of colored ion-pair complex with maximum stability and sensitivity were established by a number of preliminary experiments. Britton buffer was found to be suitable for GR method. Chloroform was preferred to other solvents (Benzene, Dichloromethane, and Diethyl ether) for its selective and quantitative extraction (Fig. 3).

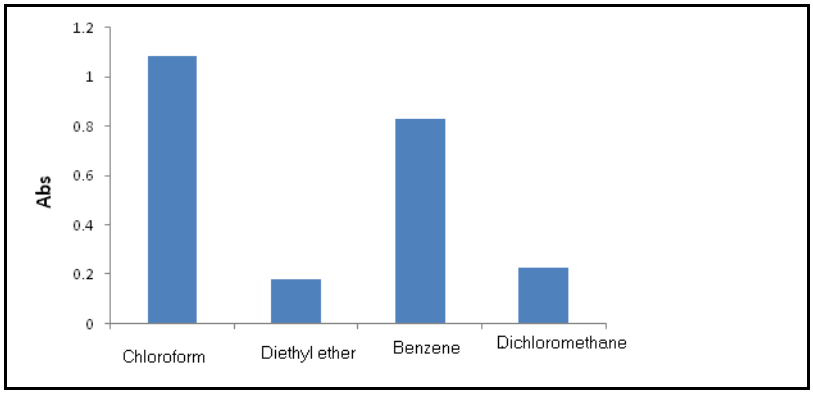


Fig 3. Effect of organic solvent on absorbance of AMB-GR.

Optimum conditions were fixed by varying one parameter at a time while keeping other parameters constant and observing its effect on the absorbance at 420 nm for GR. For GR, effect of pH was studied by extracting the colored complex species at different pH. Maximal absorbance was observed at the pH 2 (Fig 4). using 2 mL of buffer (Fig 5).

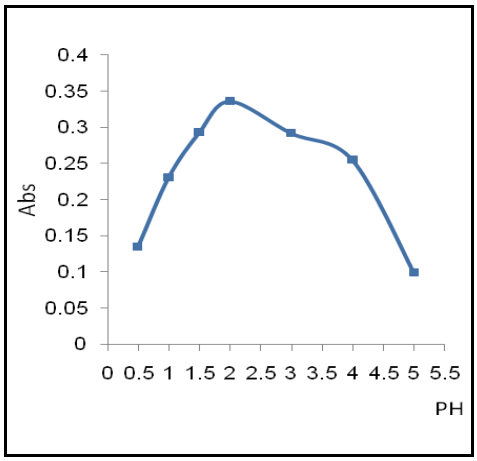


Fig 4. Effect of pH on the absorbance of AMB-GR.

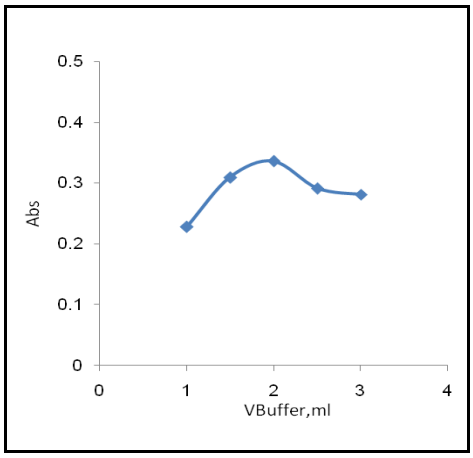


Fig 5. Effect of volume buffer on the absorbance of AMB-GR.

A volume of 3.5ml of GR(1.10^{-3}M) was found to be optimal for complete complexation, since the absorbance was found to be maximum at the mentioned volume. The effect of the reagent's concentration on the absorbance is shown in (Fig 6).

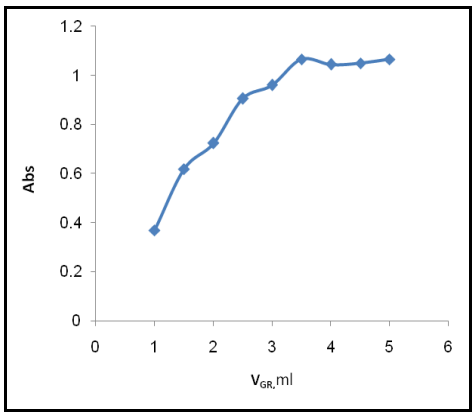


Fig 6. Effect of reagent concentration on the absorbance of AMB-GR.

Stoichiometric relationship [41]

The stoichiometric ratio of the drug to dye in the colored complex was determined using the molar ratio and continuous variation methods. The results obtained showed that a 1:1 complex was formed between amlodipine and gresol red It is apparent from the data that shown in (Fig.7 and 8).

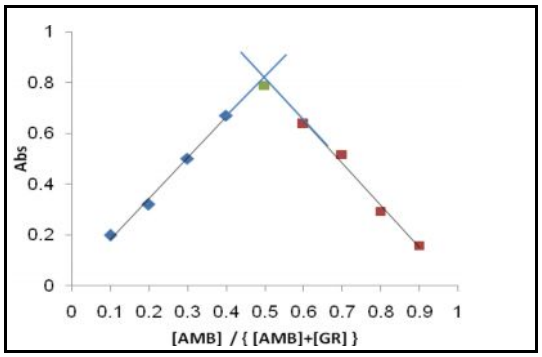


Fig 7. job's method of continuous variation of AMB-GR complex.

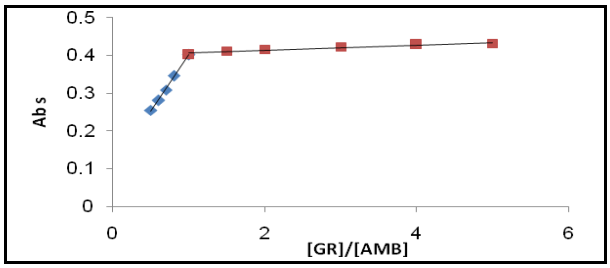


Fig 8. Mole- ratio method of AMB-GR complex.

Linearity and range [25-26]

The Beer's law range, molar absorptivity, Sandell's sensitivity, regression equation, Ringbom concentration range and correlation coefficient determined for the method are given in Table 1. A linear relationship was found between the absorbance at λ_{max} and the concentration of the drug in the range (1.1-5.5) $\mu g.ml^{-1}$ for GR method in the final measured volume of 10ml (Fig.9). Regression analysis of the Beer's law plots at λ_{max} reveals a good correlation. The graphs show negligible intercept and are described by the regression equation, A=mC+b (where A is the absorbance of 1cm layer, m is the slope, b is the intercept and C is the concentration of the measured solution in $\mu g.ml^{-1}$) obtained by the least-squares method. The high molar absorptivities of the resulting colored complexes indicate the high sensitivity of the methods.

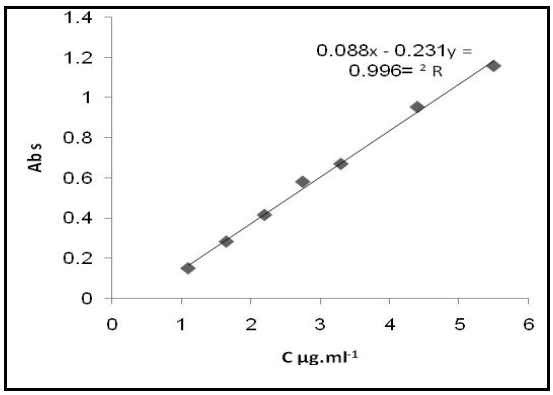


Fig 9. The linear range for determination AMB by formed complex (AMB-GR) and extraction with Chloroform

Table 1. Statistical data of the regression equations for the determination of AMB with the proposed method.

Extraction method with GR	Parameters
420	λmax,nm
1:1	Stoichiometric ratio
1.1- 5.5	Beer's law range ,($\mu g.ml^{-1}$)
1.1- 3.3	Ringbom conc. range, ($\mu g.ml^{-1}$)
0.071	Detection limit, ($\mu g.ml^{-1}$)
0.0257	Sandell's sensitivity(SS), $\mu g.cm^{-2}$
44110	, $L.mol^{-1}.cm^{-1}\varepsilon$
Regression equation[a]	
0.231	Slope
0.088 -	Intercept
$R^2 = 0.996$	Correlation coefficient

[a] With respect to A=mC+b, where C is the concentration ($\mu g.ml^{-1}$) and A is absorbance.

Validation of the method [27-28]

The validity of the method for the analysis of AMB in its pure state and in its formulations was examined by analyzing the samples using the proposed procedure. The results obtained for pure drug are given in Table 2. The precision and accuracy of the method were tested by analyzing five replicates of the drug. The low values of relative standard deviations (RSD%) indicate good precision and reproducibility of the methods. The results of analysis of dosage forms are given in Table 3. The results were reproducible with low RSD% values. The average percent recoveries obtained were quantitative (99.09%-102.42%) indicating good accuracy of the methods.

Table 2. Determination of AMB in pure form by GR method.α=95% , t = 2.776 , n = 5

C_{taken} , µg.ml^{-1}	C_{found}*, µg.ml^{-1}	R%	RSD%	$\overline{X} \pm \delta X$, µg.ml^{-1}
1.65	1.69	102.42	0.59	1.69 ± 0.01
2.2	2.18	99.09	1.83	2.18 ± 0.05
2.75	2.782	101.16	0.40	2.782 ± 0.01
3.3	3.32	100.61	1.81	3.32 ± 0.07

*Average of five determinations.

Table 3. Determination of AMB in (Amlodipine-Medico5, 5mg/tab) Tablets by GR method. α=95%, t = 2.776 , n = 5

C_{taken} , µg.ml^{-1}	C_{found}*, µg.ml^{-1}	R%	RSD%	t
2.00	2.02	100.92	1.37	1.62
3.00	3.02	100.3	1.13	1.05
4.00	3.99	99.84	084	1.14

*Average of five determinations

Application to the pharmaceutical dosage forms

The proposed method was successfully applied to determination AMB in pharmaceutical preparation (Amlodipine-Medico5, 5mg/tab). The applicability of the proposed method for the assay of the studied drug in pharmaceutical formulations was examined by analyzing various samples and the results were tabulated in Table 4. The results were reproducible with low RSD% values. The average percent recoveries were good, indicating good accuracy of the proposed method comparison to the pharmacopoeial method .

Table 4. Determination of AMB in Tablets with the proposed method and pharmacopoeia method.

Amlodipine-Medico5, 5mg/tab	pharmacopoeial method			proposed method		
	C_{found}*,mg/tab	R%	RSD%	C_{found}*,mg/tab	R%	RSD%
	5.07	101.51	0.73	5.03	100.53	1.13

*Average of five determinations

Conclusion

The proposed method is simple, accurate, precise and rapid. Therefore, this approach could be considered for the analysis of Amlodipine in the quality control laboratories. Proposed method makes use of simple reagent, which an ordinary analytical laboratory can afford. Method is sufficiently sensitive to permit determination even down to 0.071 µg.ml^{-1}. The sensitivity in terms of molar absorptivity and the precision in terms of RSD% of the method are very suitable for the determination of Amlodipine fumarate in pure and dosage forms. The commonly used additives such as starch, lactose, titanium dioxide and magnesium stearate do not interfere with the assay procedure.

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