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UV - Spectrophotometric Estimation of Amlodipine Besylate by Using Hydrotropic Solubilization with 1 M Sodium Acetate.

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ABSTRACT

Various organic solvents have been employed for the solubilization of poorly water soluble drugs to carry out spectrophotometric analysis. Drawbacks of organic solvents include their high cost, toxicity and pollution. Hydrotropy is a solubilization phenomenon of increasing the solubility of a solute by the addition of high concentrations of alkali metal salts of various organic acids such as sodium benzoate, sodium salicylate, urea, nicotinamide, sodium citrate and sodium acetate and have been observed to enhance the aqueous solubilities of many poorly water-soluble drugs. Hydrotropic solubilization may be a proper choice to preclude the use of organic solvents. A novel, simple, fast and reproducible UV spectrophotometric method was developed using 1M sodium acetate solution as hydrotropic solubilizing agent for the estimation of poorly water soluble drug amlodipine besylate. Hydrotropy exhibits absorption maximum at 366 nm. Sodium acetate did not show any absorbance above 225 nm and thus no interference in the estimation of drug was seen. Beer's law was found to be obeyed in the concentration range of 20-90µg/ml. The correlation coefficientfor amlodipine was0.99982.The results of analysis have been validated as per ICH guidelines.

Keywords: Amlodipine , Analysis, Hydrotropy ,Solubilization.

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INRODUCTION

Hypertension is one of the most commonly occurring cardiovascular risk factors. Amlodipine Besylate, a dihydropyridine calcium channel blocker, is approved for the treatment of angina pectoris. Amlodipine Besylate monohydrate is chemically 2-[(2-Aminoethoxy) methyl] -4-(2-chlorophenyl)-3-ethoxy carbonyl-5- methoxycarbonyl-6-methyl- 1,4-dihydropyridine benzene sulfonate. The chemical structure of Amlodipine Besylate is shown in Figure 1 [1]. Amlodipine has been determined by spectrophotometric methods [2], HPTLC methods [3], HPLC tandem mass spectrometric method [4] and adsorptive square wave anodic stripping voltammetry [5]. Hydrotropy is a solubilization phenomenon of increasing the solubility of a solute by the addition of fairly high concentrations of alkali metal salts of various organic acids such as concentrated aqueous solutionsof sodium benzoate, sodium salicylate, urea,nicotinamide, sodium citrate and sodium acetate and have been observed to enhance the aqueous solubilities of many poorly water soluble drugs.Maheshwari et al. has analyzed various poorly watersoluble drugs using hydrotropic solubilization phenomenon [6-12] viz. ketoprofen, salicylic acid,frusemide,cefixime,amoxicillin, hydrochlorothiazide and aceclofenac. Aqueous solubility of AML was enhanced to a great extent in 1M sodium acetate. The primary objective of the present investigation was to employ the hydrotropic solution to extract the drug from its dosage form and precludes the use of corrosive organic solvents.

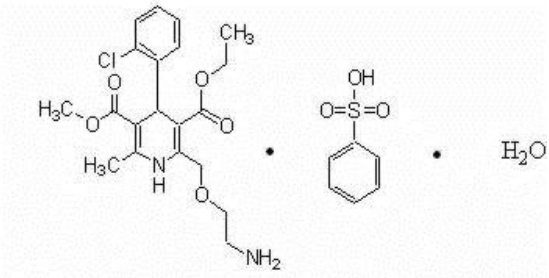

Figure 1: The chemical structure of Amlodipine Besylate

MATERIALS AND METHOD

Instrument

Spectrophotometric analysis was carried out by using a double beam UV-visible Spectrophotometer (Shimadzu model UV-1700, Japan) with 1cm matched quartz cells .

Reagents and Chemicals

Reference standard of amlodipine besylate was generous gift from Cadila Healthcare Ltd, Ankleshwar (India). All chemicals were analytical grade obtained from SD fine chemicals. Water was purified by glass distillation apparatus.

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Methods

Preliminary Solubility Study of the Drug

Solubility of amlodipine besylate was determined at 28±1°. An excess amount of drug was added to 1M sodium acetate solution in vials. The vials were shaken mechanically for 12 h at 28±1° in a mechanical shaker. These solutions were allowed to equilibrate for the next 24 hours and then centrifuged for 5 minutes at 2000 rpm. The supernatant of each vial was filtered through Whatmann filter paper No. 41. The filtrates were diluted suitably and analyzed spectrophotometrically against corresponding solvent blank.

Preparations of Standard Drug Solutions

For hydrotropic solubilization, 50 mg of pure AML was dissolved in 50 ml of 1M sodium acetate solution and stirred for 15 minutes and the final volume was made up to 100 ml with distilled water [13]. The solution was filtered through Whatmann filter paper No. 41 and was diluted with distilled water to prepare working concentrations of 500 µg/ml of AML.This stock solution was further diluted suitably with distilled water to get a concentration of 30µg/ml and was then scanned in the UV range of 200-400nm.The spectrum showed an absorption maximum at 366 nm (Figure 2). From the spectra of the drug AML and that of1M sodium acetate solution, it was found that the 1M sodium acetate solution used does not interfere with the sampling wavelength. Therefore 1M sodium acetate solution is used for the solubilization of drug.

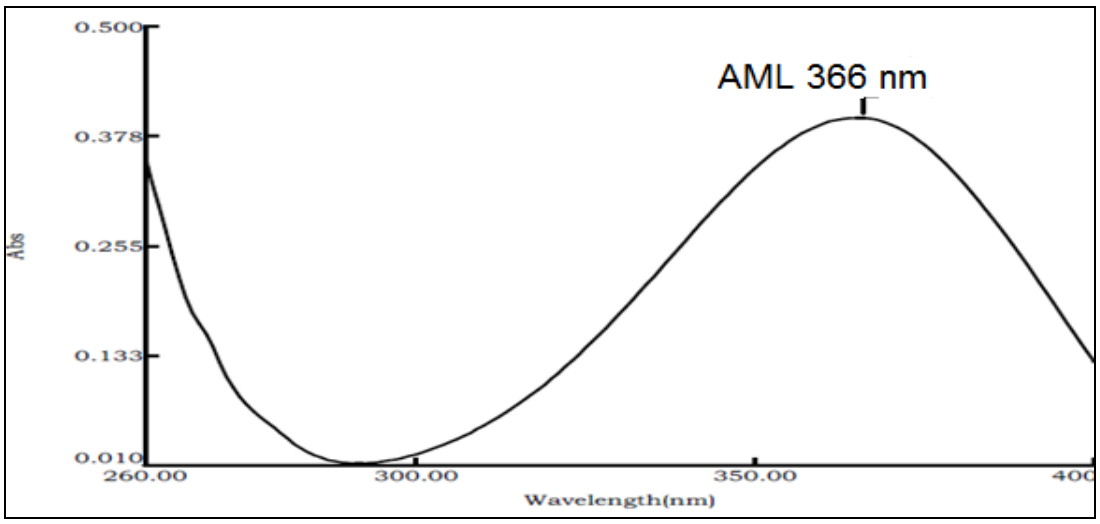

Figure 2: UV absorption spectrum of Amlodipine in 1M sodium acetate

Calibration curve of Amlodipine R.S in 1M sodium acetate solution

Aliquots of stock solutions corresponding to 20-90 µg/ml were prepared and absorbance measurements were carried out at 366 nm against the blank prepared in the same

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manner omitting the drug. Calibration curve was prepared by plotting absorbance against concentration (Figure.3) and the data is given in Table.1.

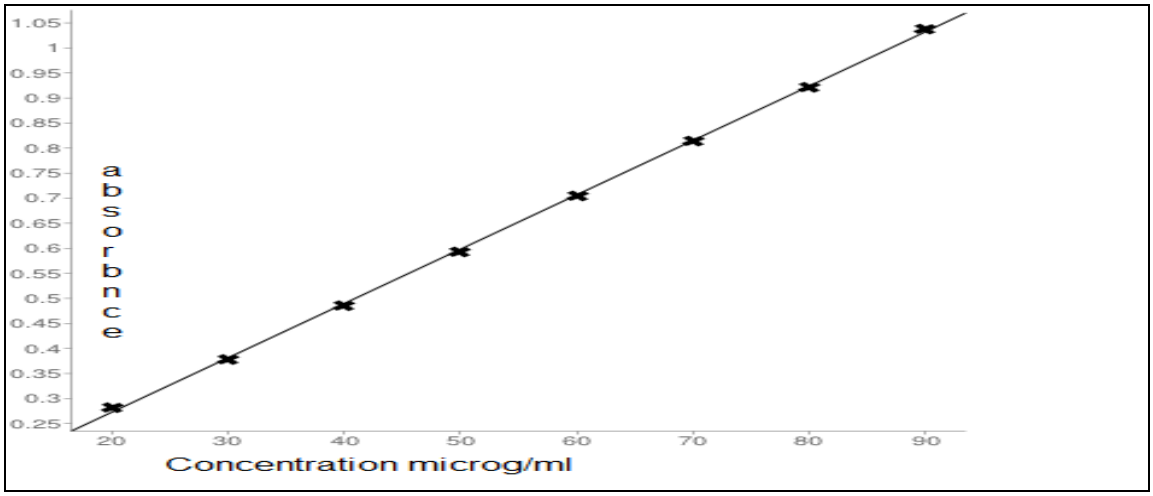

Figure 3: Calibration plot of Amlodipine R.S in 1M sodium acetate solution

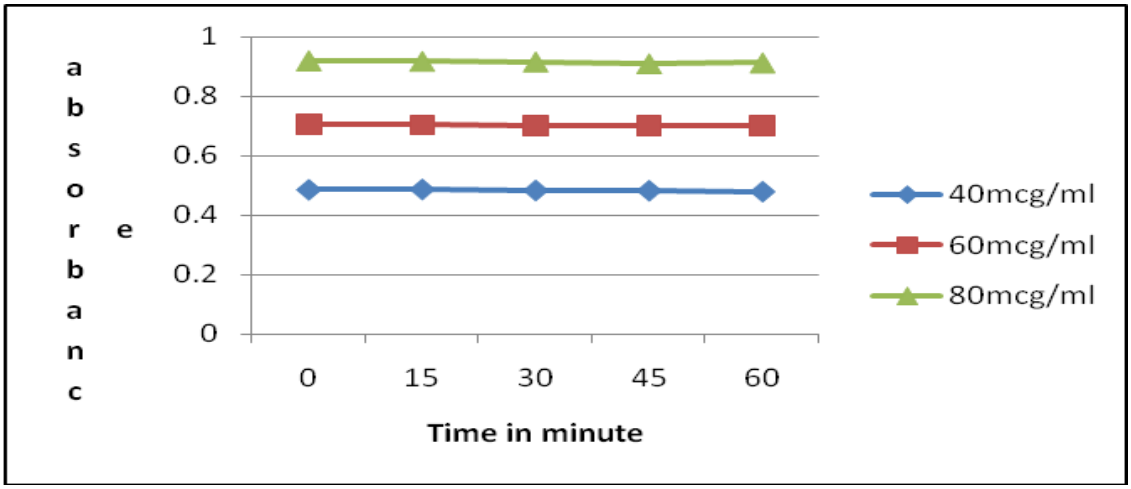

Figure 4: Stability profile of amlodipine in 1M sodium acetate

Statistical evaluation of calibration plot

The data in table (1) was used to derive a regression equation of the absorbance (Y) on the concentration (X) by the principle of least squares.

The equation is as follows

$$Y = aX + b$$

Y= 0.010846x + 0.5582

Correlation coefficient was found to be 0.99982

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The optical characteristics of Amlodipine Besylate for the developed method is furnished in table.2

Table 1: Calibration data of Amlodipine R.S in 1M sodium acetate solution

Sl No:	Volume of AML SolutionA (ml)	Concentration of AML in final solution (µg/ml)	Absorbance at 366 nm
1	0.2	20	0.282
2	0.3	30	0.378
3	0.4	40	0.486
4	0.5	50	0.593
5	0.6	60	0.705
6	0.7	70	0.815
7	0.8	80	0.922
8	0.9	90	1.038

Inference: The data reveals that Beer's law is obeyed from 20-90 µg/ml.

Table 2: Optical Characteristics of Amlodipine Besylate

No	Parameters	
1	Beers law limit	20-90µg/ml
2	Correlation coefficient	0.9982
3	Y= ax + b	Y= 0.010846x + 0.5582
4	Molar absorptivity	1.0108 X 10^4.L/mol.cm.

Table 3: Results of estimation of Amlodac tablets

Sl. No	Conc. of Amlodipine (µg/ml)	Standard Absorbance at 366nm	Sample Absorbance at 366nm	% Label claim	Active content per tablet (mg)	Average content per tablet (mg)
1	40	0.483	0.489	100.08	5.04	5.045
2	60	0.705	0.709	100.10	5.05	

Table 4: Results of recovery studies

Sl No	Brand name	AML R.S added /spiked	Total concen. found	% recovery of pure drug * (Mean±S.D) (n=3)	%RSD
1	Amlodac 5mg	5mg	10.05	100.20±0.439	0.98
		10mg	15.12	101.8±0.099	0.26
2		15mg	20.05	99.99±0.385	0.88

Table 5: Results of intraday precision study

Sl No.	Concentration (µg/ml)	Absorbance at 366 nm			RSD,%
		0 hr	1.5 hr	3hr	
1	40	0.380	0.364	0.354	1.76
2	60	0.555	0.541	0.534	1.97
3	80	0.732	0.720	0.708	1.67

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Table 6: Results of interday precision study

SI No.	Concentration (µg/ml)	Absorbance at 366nm			RSD,%
		1st day	2nd day	5th day	
1	40	0.483	0.485	0.481	0.41
2	60	0.702	0.709	0.704	0.51
3	80	0.924	0.927	0.923	0.23

Stability Profile

The period over which absorbance value at 366 nm, of Amlodipine Besylate in sodium acetate solution remained stable was investigated using three different concentrations of 40, 60, and 80 µg/ml. The absorbance values were measured at 15 min intervals for a period of 1 hour. The stability profile is shown in figure.4.

Procedure for analysis of tablet formulation:

Twenty commercially available tablets, Amlodac (Zydus Health Care) were accurately weighed and finely powdered in a glass mortar. The average weight of each tablet was calculated and were powdered finely in a glass mortar. Powder equivalent to 50 mg of AML was weighed and transferred to 100 ml volumetric flask, 70 ml of 1M sodium acetate solution was added and stirred for 15 min to dissolve the drug and the final volume was made up to 100 ml with distilled water. The solution was filtered through Whatmann fitter paper No. 41 and the first few ml were rejected. The filtrate was diluted suitably with distilled water to get 40µg/ml and 60µg/ml of amlodipine besylate. The absorbance at 366 nm was measured and the amount of drug present in the sample solutions were obtained from the slope and intercept values obtained from the calibration curve. The experiments were repeated three times to check its reproducibility. The results of analysis of tablet formulations are recorded in Table 3.

Method Validation

The method was validated according to ICH guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision, robustness and accuracy for the analyte [14] .To evaluate the validity and reproducibility of the method, known amount of pure drug was added to the analyzed sample of tablet powder and the mixture was analyzed for the drug content using the proposed method. This parameter was evaluated by the recovery studies at concentration levels of 50%, 100%, and 150% of AML which consisted of adding known amounts of Amlodipine Besylate reference materials to the samples. The percentage recovery was found to be within range (Table 4).

The intraday and interday precision study of Amlodipine was carried out by estimating the corresponding responses three times on the same day and on three different days (1st, 2nd and 5th day) for three different concentrations of Amlodipine (40, 60 and 80 µg/ml) and the results are reported in terms of relative standard deviation in table.5 and table.6

Detection limit (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

The LOD of Amodipine by the proposed method was found to be 5µg/ml.

Quantitation Limit (LOQ)

The quantitation limit of an individual analytical procedure is the lowest concentration of analyte in a sample, which can be quantitatively determined with a suitable level of precision and accuracy.The LOQ of Amodipine by the proposed method was found to be 10 µg/ml.

Linearity

The linearity of an analytical procedure is its ability, within a given range to obtain test results that are directly proportional to the concentration of analyte in the sample. The calibration curve of Amodipine was linear over the range of 20-90µg/ml.

DISCUSSION

Quantitative estimation of poorly water-soluble drugs involve the use of organic solvents. In the present investigation, hydrotropic solubilization is employed to enhance the aqueous solubility of poorly water-soluble drugs like Amlodipine Besylate in tablet dosage forms. The results of solubility studies indicated that enhancement in the aqueous solubility of Amlodipine Besylate in 1M sodium acetate solution was more than 5 folds as compared to their solubility in distilled water. Therefore, this solution was employed to extract Amlodipine Besylate from the fine powder of tablet formulation. By proper choice of hydrotropic agents, the use of organic solvents in analysis may be discouraged to a large extent. It is evident that there is good agreement between the amounts estimated and those claimed by the manufacturers. The mean percentage label claim 100.10 was found for Amlodac which is very close to 100 with low values of standard deviation which confirms the accuracy of the proposed method. Accuracy, reproducibility and precision of the proposed method were further confirmed by the mean percentage recovery values (99.99% to 101.80%), which were close to 100 with low values of standard deviation .The proposed method for determination of AML showed molar absorptivity of 1.0108 X 10^4/mol.cm. Linear regression of absorbance on concentration gave the equation Y= 0.010846x + 0.5582with a correlation coefficient (r) of 0.9982.

CONCLUSION

The statistical analysis of the results revealed that the developed method is economic, simple, precise and rapid. Hence it can be employed for the routine analysis for the estimation of amlodipine from marketed formulations and in biological fluids.

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