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Qualitative and Quantitative Analysis on Some Cardiovascular Drugs

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In this present paper, qualitative and quantitative analysis were made on some cardiovascular drugs *viz.*, amlodipine besylate, indapamide, atenolol and clopidogrel bisulphate by UV-Visible spectroscopy. The UV-Visible spectral recordings were carried out over ranges 200-400 nm. The stability of the drug under different environmental conditions is one of the quality assurance methods undertaken in the pharmaceutical laboratory. The UV-visible spectra have been recorded for the drug kept in suitable storage condition and for that exposed to various environmental hazards. The sets of internal standards of these drugs are compared with suitable storage conditions to check whether any change has taken place due to the exposure. The method of assay is experimented with these drugs using UV-visible spectroscopic measurements.

Key Words: UV-Visible spectroscopy, Cardiovascular diseases, Amlodipine besylate, Atenolol, Clopidogrel bisulphate, Indapamide.

INTRODUCTION

To investigate the structure and analysis of some pharmaceutical and biological active compounds, spectroscopic techniques have been widely used in the recent past. Gunasekaran *et al.*[1,2] have done qualitative analysis on various pharmaceutical drugs, using IR and UV-Visible spectral measurements. By keeping all these factors, in the present investigation a qualitative and quantitative analysis have been made on some cardiovascular drugs.

Cardiovascular drugs are used in the treatment of diseases of heart and blood vessels. Different kinds of drugs included in this category are cardiotonoic drugs[3] such as cardiac glycosides, antihypertension drugs, antiarrhythemic drugs, anti-anginal agents, vasodilators, lipid lowering agents, *etc*. The drugs chosen for present study falls on various above categories. Amlodipine besylate, atenolol, indapamide and clopidogrel bisulphate are some cardiovascular drugs chosen for the present study.

Chen *et al.*[4] has done clinical investigation on amlodipine pharmacokinetics, to develop a sensitive and specific mass spectrometric method for determination of amlodipine in human plasma. Nadazdin and Devies[5] investigated therapeutic mechanisms of atenolol and diltiazem in patients with variable-threshold angina. Vukovich[6]

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have done a multi-centre clinical investigation for demonstrating the effectiveness and tolerance of indapamide for the treatment of hypertension. Thomas *et al.*[7] has studied aspirin and clopidogrel bisulphate resistance in patients with diabetes mellitus. Though investigations on these cardiovascular drugs have been made by many researchers, but not much work is done on these said drugs spectroscopically. Hence the present study aims to make use of UV-Visible spectroscopic method for the qualitative and quantitative analysis of these drugs. The pharmaceutical data of these drugs are presented in Table-1 and their molecular structures are shown in Figs. 1 and 2.

TABLE-1
PHARMACEUTICAL DATA OF CARDIOVASCULAR DRUGS

Characters	Amlodipine besylate	Atenolol	Clopidogrel bisulphate	Indapamide
m.f.	$C_{26}H_{31}N_2O_8SCl$	$C_{14}H_{22}N_2O_3$	$C_{16}H_{18}NO_6S_2Cl$	$C_{16}H_{16}N_3O_3SCl$
Solubility (mg/L)	75.3	13.5	50.78	75
m.p. (ºC)	178-179	146-148	158	161
Category	Anti hypertensive agent Calcium channel blocker	Antiarrhythmic agent	Antiplatelet agent	Anti hypertensive agent Diuretics

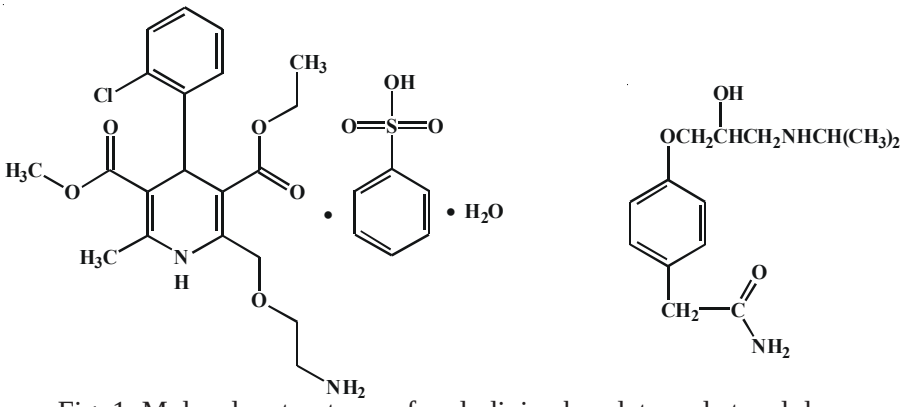

Fig. 1. Molecular structures of amlodipine besylate and atenolol

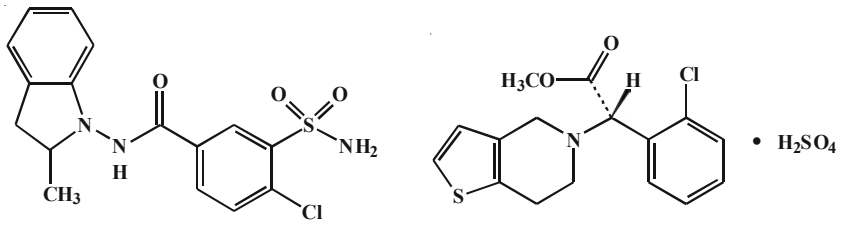

Fig. 2. Molecular structures of indapamide and clopidogrel bisulphate

EXPERIMENTAL

The spectroscopic pure samples of amlodipine besylate, atenolol, indapamide and clopidogrel bisulphate were procured from Orchid Chemicals and Pharmaceuticals Ltd., India and used as such. The UV-Visible spectra were measured using Shimadzu UV1601 spectrophotometer over the region 200-400 nm with fast scan speed in absorbance measuring mode, with fixed sampling interval 0.2 and slit width 2.0 (nm). The experiment was carried out at Dr. Ceeal laboratorary, C.L. Baid Mehta College of Pharmacy, Chennai, India.

Beer-Lambert law

The UV-visible spectral measurements are carried out on amlodipine besylate and by checking the sample obeying Beer's law, the absorption peaks are identified. The sample is dissolved in water and a stock solution is prepared. Thus, the drug is made into solution suitable for UV absorption and UV-Visible spectral readings were carried out using Shimadzu UV-1601 spectrophotometer. The sample shows absorption peaks at 366 and 239 nm. The spectral measurements were carried out for different concentrations (10, 15, 20, 25, 30 mcg/mL) of the drug. The UV-Visible spectra of amlodipine besylate at different concentrations are shown in the Fig. 3. It is found that as the concentration increases, the absorption level increases satisfying Beer's law. The linearity curve, verifying Beer's law for the drug amlodipine Besylate is also shown in Fig. 3 and the linearity coefficient found to be 0.9998, which is used as a parameter to identify the quality of the drug amlodipine besylate.

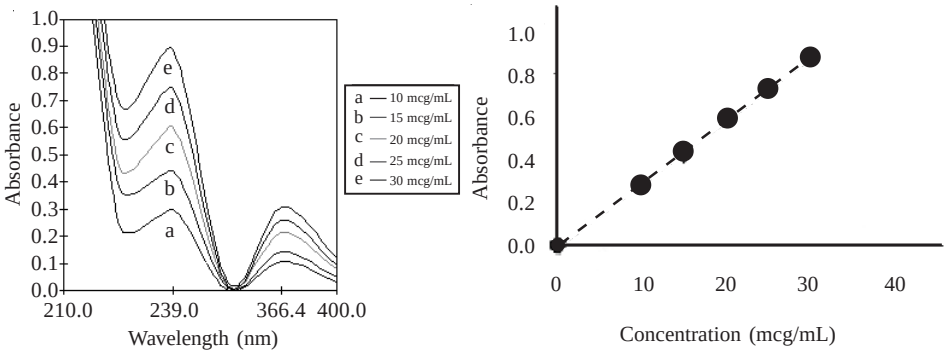

Fig. 3. Overlaid spectra at different concentrations and linearity curve of amlodipine besylate

The above procedure is repeated for atenolol, indapamide and clopidogrel bisulphate, where methanol was used as a solvent. The sample of atenolol shows absorption peaks at 283 and 276 nm. Fig. 4 shows spectra of drug at various concentrations (100, 125, 150, 175, 200 mcg/mL) and the linearity curve, verifying Beer's law for the drug atenolol. The linearity coefficient is found to be 0.9996.

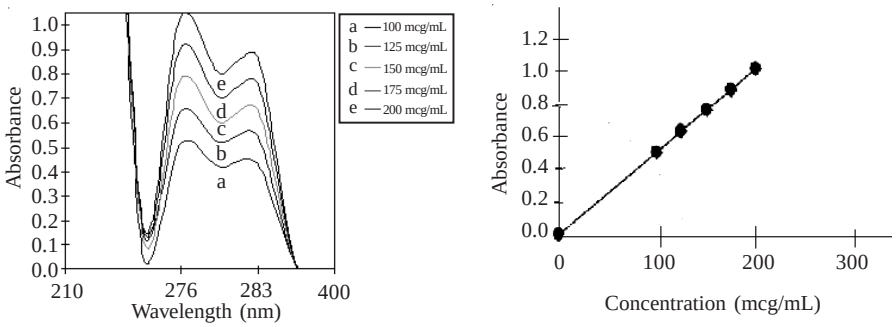

Fig. 4. Overlaid spectra at different concentrations and linearity curve of atenolol

The sample of indapamide shows absorption peaks at 279 nm and 240 nm. Fig. 5 shows spectra of drug at various concentrations (5, 7.5, 10, 12.5, 15 mcg/mL) and the linearity curve, verifying Beer's law for the drug indapamide. The linearity coefficient is found to be 0.9999.

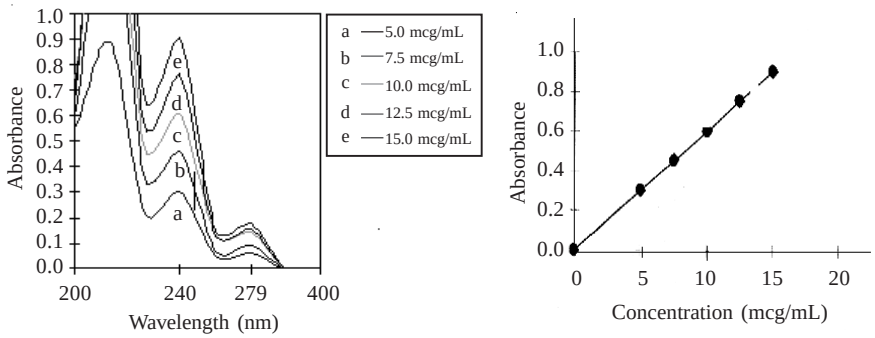

Fig. 5. Overlaid Spectra at different concentrations and linearity curve of indapamide

The sample of clopidogrel bisulphate shows absorption peaks at 278, 270 and 264 nm. Fig. 6 shows spectra of drug at various concentrations (25, 50, 75, 100, 125, mcg/mL) and the linearity curve, verifying Beer's law for the drug clopidogrel bisulphate. The linearity coefficient is found to be 0.9922.

Study of storage condition

Among the different quality assurance measures used in the control of manufacturing and formulation of drugs, two parameters are important which is the check on the shelf life of the drugs, stability under different storage conditions which has to be tested at every stage. Hence during the fabrication of the drugs, the various raw materials that are used in the fabrication of the drugs should undergo a rigorous qualitative test. Spectroscopic technique, UV-Visible bands were made to study the quality of the drugs under different storage conditions.

The ideal storage condition (ISC) for the drugs used in the present study should be stored in airtight containers, at temperatures between 59 and 86 ºF (15 and 30 ºC) is permitted. Store away from heat and light. Do not store in moisture place.

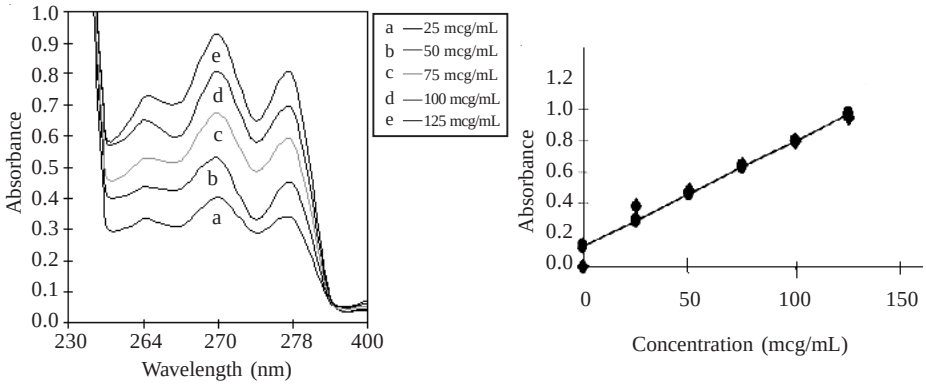

Fig. 6. Overlaid spectra of clopidogrel Bisulphate at different concentrations and linearity curve of clopidogrel bisulphate

Three sets of equal amount of amlodipine besylate in the powder form (each 25 mg) have been taken for the investigation. One set of the drug was stored at room temperature *i.e.*, at ideal storage condition (ISC), while another was stored at cold condition (ice point), the third set of the drug was exposed to sunlight continuously for a stipulated period of 4 h to make an internal standard calculation on the compound amlodipine besylate.

UV-Visible spectral investigation has been carried out to study the variations in the absorbance of λ_{max} in amlodipine besylate at different storage conditions. The samples of amlodipine besylate are exposed to different storage conditions *viz.*, ideal storage condition, cold condition and sunlight. Overlaid UV-Visible spectra of amlodipine besylate (at 20 mcg/mL concentration) at different storage condition and bar chart shows the variance of λ_{max} with absorbance at different storage condition for amlodipine besylate is shown in Fig. 7. From this figure, it is observed that the absorbance of the drug kept at ideal storage condition varies with the absorbance of the drug placed in the sunlight and at the ice point. Hence it can be concluded that the drug under study is to be stored in ideal storage condition to retain its pharmaceutical properties. Similarly, the experiment was performed for atenolol (at 150 mcg/mL concentration), indapamide (at 10 mcg/mL concentration) and clopidogrel bisulphate (at 75 mcg/mL concentration). The overlaid UV-visible spectra of atenolol, indapamide and clopidogrel bisulphate and corresponding bar chart at different storage conditions are presented in Figs. 8-10, respectively. The internal standard ratio among the absorbance of wavelength maxima are calculated and the sets of internal standards of these drugs stored under different storage conditions are compared with that of the drugs stored under ideal storage condition to check whether any change in the light absorption characteristics of the drugs has taken place. From the Tables 2-5, it is observed that the internal standard calculation for the various storage conditions showed significant change with the drug kept in ideal storage condition. It is observed that the drug activity changes more significantly due to improper storage of the drugs.

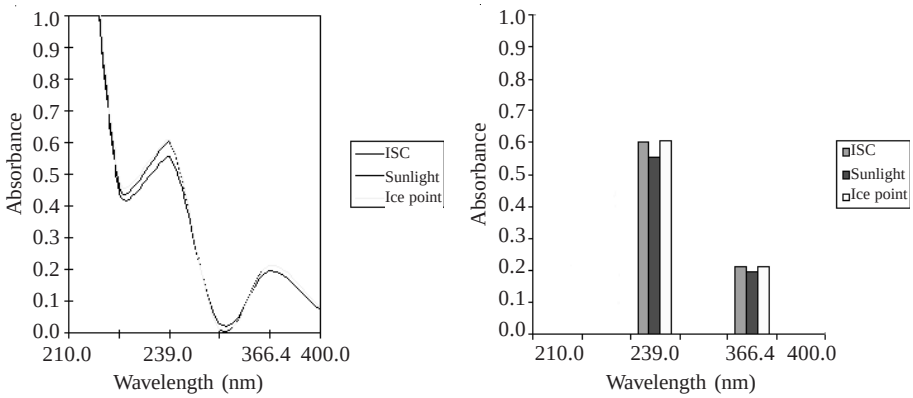

Fig. 7. Overlaid spectra at different storage condition and bar chart for the variance of λ_{max} with absorbance at different storage condition for amlodipine besylate

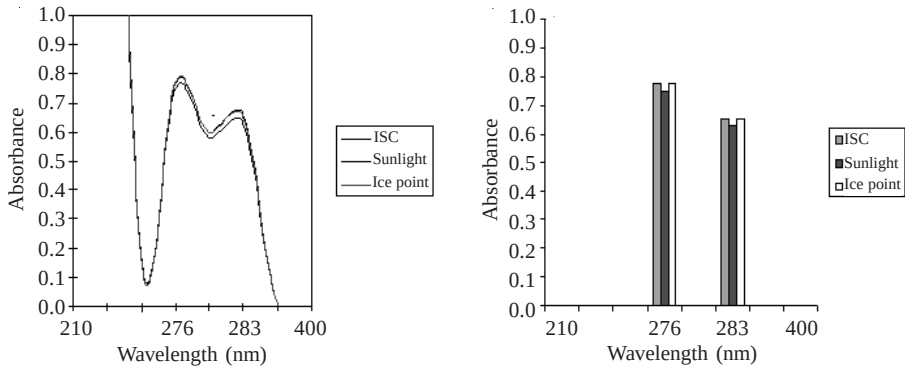

Fig. 8. Overlaid spectra at different storage condition and bar chart for the variance of λ_{max} with absorbance at different storage condition for atenolol

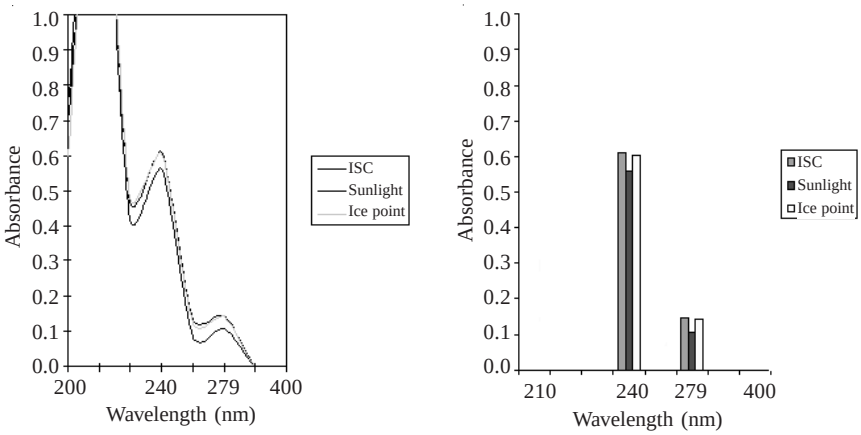

Fig. 9. Overlaid spectra at different storage condition and bar chart for the variance of λ_{max} with absorbance at different storage condition for indapamide

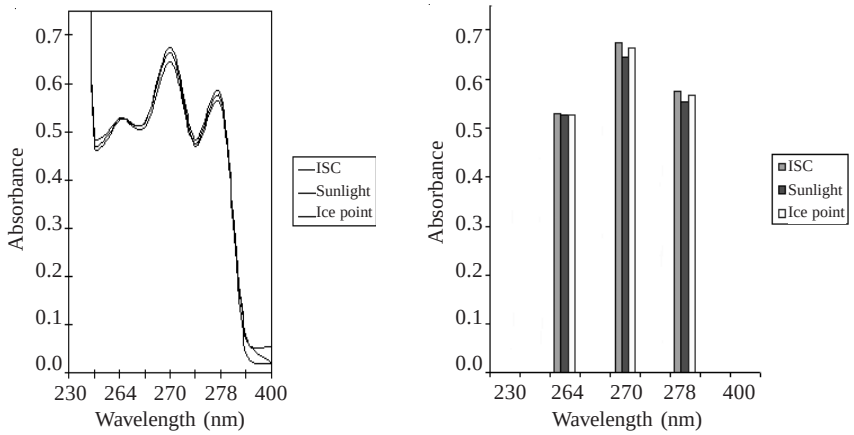

Fig. 10. Overlaid spectra at different storage condition and bar chart for the variance of λ_{max} with absorbance at different storage condition for clopidogrel bisulphate

TABLE-2
INTERNAL STANDARD CALCULATION OF AMLODIPINE BESYLATE

Storage conditions	A366/A239
Ideal storage condition	0.4269
Sunlight	0.3404
Cold condition	0.3500

TABLE-3
INTERNAL STANDARD CALCULATION OF ATENOLOL

Storage conditions	A282/A276	A282/A230	A276/A230
Ideal storage condition	0.8400	0.254	0.303
Sunlight	0.8389	0.216	0.257
Cold condition	0.8380	0.218	0.261

TABLE-4
INTERNAL STANDARD CALCULATION OF INDAPAMIDE

Storage conditions	A279/A240	A279/A205	A240/A205
Ideal storage condition	0.1936	0.052	0.2705
Sunlight	0.1947	0.069	0.3545
Cold condition	0.2370	0.084	0.8760

TABLE-5
INTERNAL STANDARD CALCULATION OF CLOPIDOGREL BISULPHATE

Storage condition	A278/A270	A278/A264	A278/A240	A270/A264	A264/A240
Ideal storage condition	0.837	1.010	0.132	1.206	0.1300
Sunlight	0.858	1.047	0.168	0.219	0.1608
Cold condition	0.852	1.085	0.170	1.273	0.1570

Assay of drugs

Assay is the estimation of potency of an active principle in a unit quantity of the preparation. The potency is the measure of the biological activity of a drug. In present study, UV-visible spectrophotometric technique is used to study the assay of drugs. Tablets remain more popular dosage form because of the advantages offered both to the manufacturer in terms of simplicity and economy of preparation, stability and convenience in packaging, transporting and dispensing and to the patient for accuracy of dosage, compactness, portability, blandness of taste and ease of administration. In the present work, medicines of amlodipine besylate, atenolol, indapamide and clopidogrel bisulphate in the form of tablets are subjected for the quantitative estimation of the drug substance in the tablet. According to Indian Pharamacopoeia the method of assay is by different methods in each of the above tablets. As a model experiment to understand how UV-Visible spectroscopic technique is employed in the assay, this method is applied in amlodipine besylate, atenolol, indapamide and clopidogrel bisulphate to estimate the active substance in the tablet.

The tablets containing amlodipine besylate as the active ingredient is obtained from a leading pharmaceutical company labeled, as amlan 5 mg. Similarly the tablets containing atenolol alone as active substance are obtained which is atol 100 mg. For indapamide the tablet indap 2.5 mg is used for assay. In the case of clopidogrel bisulphate a tablet having strength 75 mg (Pidlet 75 mg) is used for the analysis. The UV-visible spectra are recorded for all the samples in the pure form and for the tablets. By comparing the absorbance in the pure and tablet form of the sample, the quantitative estimation of the drug can be estimated. Quantitative spectrometry is an extension of calorimetry and many pharmacopoeial substances are assayed spectrophotometrically. A solution of the test substances is made at a known concentration in a suitable solvent. The absorbance is noted at a selected wavelength which is preferably that of wavelength maxima having a fairly broad, flat-topped peak. Then by making a parallel determination of a solution prepared from a pure reference sample for the same concentration, the amount of active ingredient in the test substance is calculated.

All the measurements are based on the fundamental law of spectrophotometry (*i.e.*) Beer-Lambert's law which is stated as $A = \log (I/I_0) = \alpha bc$ where, I_0 is the intensity of the incident monochromatic beam which emerges with intensity I through a solution of path length of 'b' having concentration 'c' and 'α' is the absorbance. In drug analysis, the determination of the drug content is carried out by preparing a stock solution of the test sample and the solution is diluted to the same concentration as that of the standard sample and the absorbance of the resulting solution is measured. The drug content of the tablet is calculated from Beer's law as

$$\text{Drug content of the tablet or assay} = \frac{\text{Test absorption}}{\text{Standard absorption}} \times \frac{\text{Standard weight}}{\text{Test weight}} \times \text{Average weight of one tablet}$$

In the present work, a single component system is chosen for the assay. From the spectroscopic point of view, a single component system is the one for which, at the wavelength selected for the measurement, the determination of the analyte is not influenced either by another substance or by background absorption.

Amlodipine besylate: To calculate the assay of the amlodipine besylate tablets, the tablets containing the strength of the pure drug is employed in the work which is amlan 5 mg manufactured by the same pharmaceutical company. To record UV-visible spectra, stock solution is prepared by dissolving pure amlodipine besylate in distilled water. The stock solution is further diluted and UV-visible spectra is recorded at concentration 20 mcg/mL. The spectra allows a strong absorption peak at 239 and 366 nm and the absorbance is noted. For doing the test absorption, 20 tablets of amlan (5 mg) are weighted and the average weighted of one tablet is found to be 108.95 mg. The tablet is powdered well and the powder is dissolved in distilled water to prepare a stock solution and the solution is filtered and diluted so that the concentration of the stock solution is 20 mcg/mL, to have the same concentration of that of the pure sample. The UV-visible spectra are recorded and absorbance is noted (Fig. 11). The quantitative estimation or assay of the active substance amlodipine besylate is estimated in tablet (amlan 5 mg) and is found to be 5.026 mg.

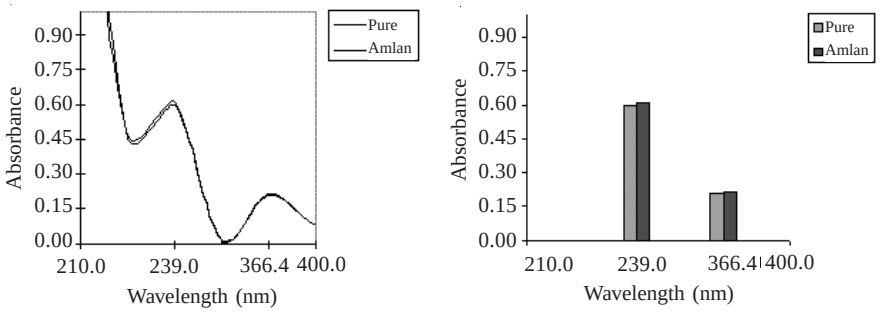

Fig. 11. Overlaid spectra and bar chart for the variance of λ_{max} with absorbance of pure amlodipine besylate and amlan tablet

Atenolol: To calculate the assay of the atenolol tablets, the tablets containing the strength of the pure drug is employed in the work which is atol 100 mg manufactured by the same pharmaceutical company. To record UV-visible spectra, stock solution is prepared by dissolving pure atenolol in methanol. The stock solution is further diluted and UV-visible spectra is recorded at concentration 150 mcg/mL. The spectra allows a strong absorption peak at 276 and 283 nm and the absorbance is noted (Fig. 12). For doing the test absorption, tablets of atol (100 mg) are weighed and the average weight of one tablet is found to be 221.85 mg. The tablet is powdered well and the powder is dissolved in methanol to prepare a stock solution and the solution is filtered and diluted so that the concentration of the stock solution is 150 mcg/mL, to have the same concentration of that of the pure sample. The UV-visible spectra are recorded and absorbance is noted. The quantitative estimation or assay of the active substance atenolol is estimated in tablet (atol 100 mg) and is found to be 100.29 mg.

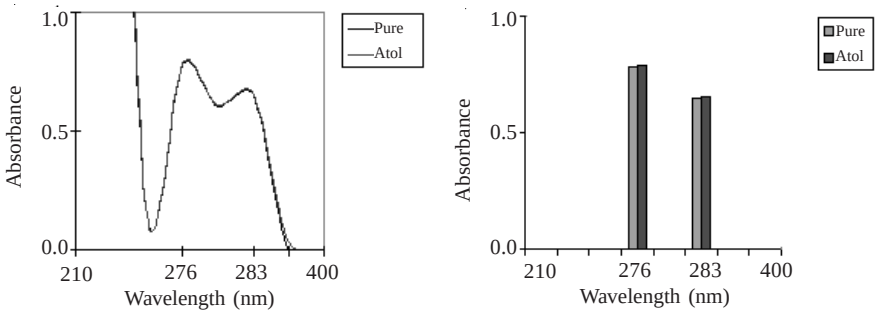

Fig. 12. Overlaid spectra and bar chart for the variance of λ_{max} with absorbance of pure atenolol and atol tablet

Indapamide: To calculate the assay of the indapamide tablets, the tablets containing the strength of the pure drug is employed in the work is indap 2.5 mg manufactured by the same pharmaceutical company. To record UV-visible spectra, stock solution is prepared by dissolving pure indapamide in methanol. The stock solution is further diluted and UV-visible spectra is recorded at concentration 10 mcg/mL. The spectra allows a strong absorption peak at 240 and 279 nm and the absorbance is noted (Fig. 13). For doing the test absorption, tablets of indap (2.5 mg) are weighed and the average weight of one tablet is found to be 304.26 mg. The tablet is powdered well and the powder is dissolved in methanol to prepare a stock solution and the solution is filtered and diluted so that the concentration of the stock solution is 10 mcg/mL, to have the same concentration of that of the pure sample. The UV-visible spectra are recorded and absorbance is noted. The quantitative estimation or assay of the active substance indapamide is estimated in tablet (indap 2.5 mg) and is found to be 2.45 mg.

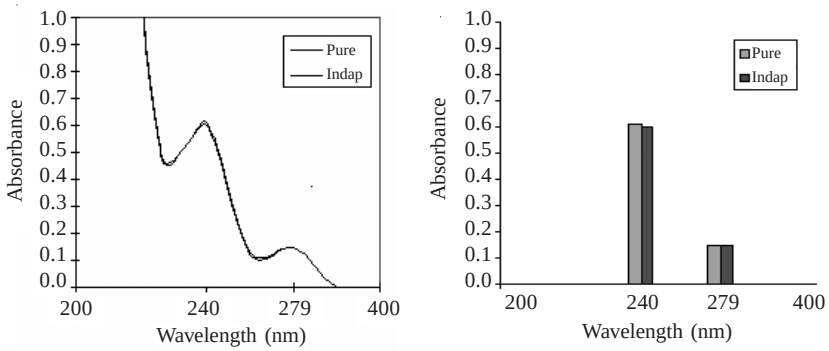

Fig. 13. Overlaid spectra and bar chart for the variance of λ_{max} with absorbance of pure indapamide and indap tablet

Clopidogrel bisulphate: To calculate the assay of the clopidogrel bisulphate tablets, the tablets containing the strength of the pure drug is employed in the work is pidlet 75 mg manufactured by the same pharmaceutical company. To record UV-visible spectra, stock solution is prepared by dissolving pure clopidogrel bisulphate in methanol. The stock solution is further diluted and UV-visible spectra is recorded

at concentration 75 mcg/mL. The spectra allows a strong absorption peak at 264, 270 and 278 nm and the absorbance is noted (Fig. 14). For doing the test absorption, tablets of pidlet (75 mg) are weighed and the average weight of one tablet is found to be 304.3 mg. The tablet is powdered well and the powder is dissolved in distilled water to prepare a stock solution and the solution is filtered and diluted so that the concentration of the stock solution is 75 mcg/mL, to have the same concentration of that of the pure sample. The UV-visible spectra are recorded and absorbance is noted. The quantitative estimation or assay of the active substance clopidogrel bisulphate is estimated in tablet (pidlet 75 mg) and is found to be 74.90 mg.

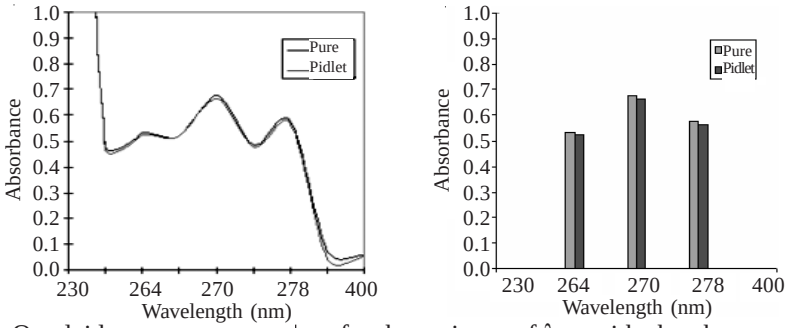

Fig. 14. Overlaid spectra and bar chart for the variance of λ_{max} with absorbance of pure clopidogrel bisulphate and pidlet tablet

Conclusion

The quantitative analysis of the drugs amlodipine besylate, atenolol, indapamide and clopidogrel bisulphate has been done by UV-visible spectral measurements. The internal standard calculation has been made for different absorbance value for the drugs exposed to different environment conditions. The method of assay has been employed in four tablets amlan, atol, indap and pidlet. In amlodipine besylate tablet (amlan 5 mg) the experimental determination is found to be 5.026 mg. In atol 100 mg, the active substance present is calculated as 100.29 mg. In indapamide, indap 2.5 mg is used for which, the UV-visible experimental determination of assay is found to be 2.45 mg. Pidlet 75 mg is used for the assay estimation in clopidogrel bisulphate and the experimental value is found to be 74.90 mg.

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