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# Development and Validation of Stability Indicating UV-VIS Spectrophotometric Method for Estimation of Acyclovir in Bulk Drug and Pharmaceutical Dosage Form

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## ABSTRACT

The simple, accurate, precise, sensitive, specific and cost- effective UV-VIS Spectrophotometric method was developed for the estimation of acyclovir in bulk and pharmaceutical dosage form, used as an antiviral drug. The absorption maxima of the drug were found to be 252.6nm. in 0.1N $H_2SO_4$solvent. The method was validated as per the ICH Guidelines. A linear response was observed in the range of 2-10µg/ml with a regression coefficient of 0.999. The developed method was precise; the % RSD was found to be less than 2%. The LOD & LOQ were found to be 2.3 and 7.0µg/ml respectively. The standard drug was subjected to acidic, alkaline, oxidative, thermal & photolytic stress degradation conditions. The samples so generated were used for degradation studies using the stability indicating UV-Visible Spectrophotometric method. This method was used for the determination of Acyclovir in tablet and cream formulation without interferences of the excipients, this indicated specificity of method.

**Keywords:** Acyclovir, stress degradation conditions, UV-VIS Spectrophotometer, ICH guidelines-validation.

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## INTRODUCTION

Acyclovir, 2-amino-9-[(2-hydroxyethoxy) methyl]-6, 9-dihydro-3H-purin-6-one) (Figure 1), is an antiviral drug, used in the treatment of chicken pox, genitical virus and shingles. It is a prototype of the group of viral agents that are activated by viral thymidine kinases (Tk) to become inhibitors of viral DNA polymerases and block viral DNA synthesis. The dose is taking with or without food. The dose is for genital herpes virus is by oral route are 200mg every 4hrs and for chicken pox 800mg 4 times daily by I.V. route 5-10mg/kg every 8 hrs for 7-10 days. Acyclovir is a white, crystalline powder with the molecular formula C8H11N5O3 and a molecular weight of 225gm. The drug is insoluble in water and it is soluble in acid and base solvent. Zovirax is the brand name for acyclovir, a synthetic nucleoside analogue active against herpes viruses. Literature survey indicated that acyclovir is official in IP 2014[1].Estimation of acyclovir was done by using UV spectrophotometric method[2] as well as stability indicating HPLC method [3]has been developed on acyclovir in bulk drug and pharmaceutical dosage forms. This research paper reported validated stability-indicating UV Spectrophotometric method for determination of acyclovir in bulk drug and their formulation. The developed stability indicating UV-Spectrophotometric method is precise, specific and sensitive similar to chromatographic HPLC method but advantage of stability indicating UV Spectrophotometric method over chromatographic HPLC method is that the developed method was simple and cost effective to check the purity of drug with time and change in temperature. The method was validated in compliance with ICH guidelines[7,8,9]. The International conference on Harmonization (ICH) guideline entitled "stability testing of new drug and Products" requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. It was thought necessary to study the stability of acyclovir under acidic, oxidative, photolytic and dry heat conditions by using simple UV-Visible spectrophotometric method.

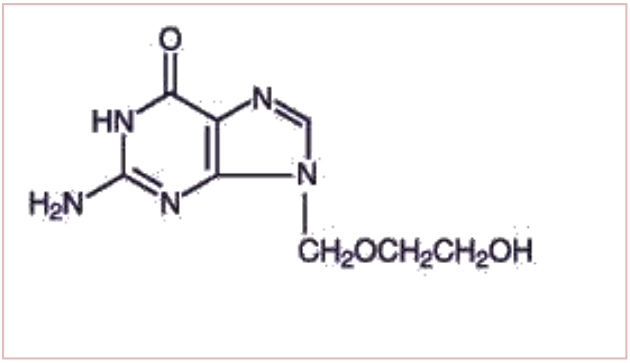



**Figure 1 Chemical Structure of Acyclovir**

# MATERIAL AND METHOD

## Apparatus

The instrument used for the study was an UV-VIS double beam Spectrophotometer (Shimadzu Model No. 1800) with spectra width of 2nm, and 1cm matched pair quartz cells was used to measure absorbance of all solutions. The scanning speed was fast and wavelength range was 200-400nm.

## Reagents and Materials

The solvent used was distilled water, 0.1 N sulphuric acid and 0.1 N sodium hydroxide AR grades.

## Preparation of stock solution

Acyclovir is insoluble in water, so standard stock solution of Acyclovir was prepared by dissolving 10mg drug in 0.5ml of 0.1N $H_2SO_4$ and solution was neutralized by 0.5 ml of 0.1N NaOH and volume made to 10 ml with of distilled water to produce a concentration of 1000µg/ml. 1ml of stock solution was taken and then diluted upto 10 ml by using distilled water to produce a concentration of 100µg/ml which is used as standard stock solution.

## Methodology

The standard solution of 10µg/ml solution was prepared and scanned in UV-VIS Spectrophotometer in the range 200-400nm using water as a blank solvent and the wavelength corresponding to maximum absorbance ($\lambda_{max}$) was found to be 252.6nm.

## Stress degradation studies of Acyclovir

The acyclovir was subjected to various stress degradation conditions, acidic, oxidative, photolytic and dry heat conditions.

## Acid hydrolysis

10µg/ml of standard acyclovir drug solution was subjected to acid hydrolysis in 0.1N HCl at room temperature for 24 hours and scanned in UV-VIS Spectrophotometer in the range 200-400nm, the change in wavelength corresponding to maximum absorbance ($\lambda_{max}$-252.6nm) was observed.

## Base hydrolysis

10µg/mlof standard acyclovir drug solution was subjected to base hydrolysis in 0.1N NaOH at room temperature for 2 hours and scanned in UV-VIS Spectrophotometer in the range 200-400nm, the change in wavelength corresponding to maximum absorbance ($\lambda_{max}$-252.6nm) was observed.

## Oxidative degradation

10µg/ml of standard acyclovir drug solution was subjected to oxidative hydrolysis in 3%$H_2O_2$at room temperature for 1 hours and scanned in UV-VIS Spectrophotometer in the range 200-400nm, the change in wavelength corresponding to maximum absorbance ($\lambda_{max}$-252.6nm) was observed.

**Thermal degradation**

The 10mg of acyclovir drug was subjected to thermal degradation in oven at 80$^{o}$Cfor 24 hours and then 10µg/ml of drug solution prepared and scanned in UV-VIS Spectrophotometer in the range 200-400nm, the change in wavelength corresponding to maximum absorbance ($\lambda_{max}$-252.6nm) was observed.

**Photo degradation**

10µg/ml of standard acyclovir drug solution was subjected to photo degradation under UV light for 24 hours and scanned in UV-VIS Spectrophotometer in the range 200-400nm, the change in wavelength corresponding to maximum absorbance ($\lambda_{max}$-252.6nm) was observed.

**Method Validation**

The method was validated as per ICH (International Conference of Harmonization) guideline for different parameters like Linearity, Accuracy, Precision, Specificity, Robustness, LOD and LOQ.

**Linearity**

Working standard solution of 2,4,6,8 and 1010µg/ml were prepared from a standard stock solution of 100µg/ml. The calibration curve of absorbance vs concentration was plotted. The drug obeys the Beer's Law with $R^2$ value 0.997.

**Precision**

The Precision of the method was checked by repeated scanning and measurement of absorbance solution without changing the parameter. In Intraday precision, three different solution of same concentration that is 10µg/ml were prepared and analyzed three times in a day i.e. morning, afternoon and evening and the absorbance's were noted. In the Interday variation study, solutions of same concentration 10µg/ml were prepared and analyzed three times for three days and the absorbance's were noted. The results were reported in terms of %RSD.

**Accuracy**

The accuracy of the method was determined by standard addition method. Preparing standard solutions of three different concentrations that is 8, 10, 12 µg/ml in which the fixed amount of 10 µg/ml solution of marketed tablet formulation was mixed and absorbance of spiked solutions was measured three times and % recovery of acyclovir was calculated.

**Robustness**

Robustness of the method was determined by making small change in set parameter that is scanning speed and wavelength range was changed and 10µg/ml of standard drug solution scanned to measure its absorbance repeatedly to check the precision of method.

**Limit of Detection**

The Limit if detection was determined by using formula 3.3 σ/S, where σ = Standard deviation of the response (y- intercept) and S = Slope of the calibration curve.

**Limit of Quantification**

The Limit if detection was determined by using formula 10 □/S.D. where σ = Standard deviation of the response (y- intercept) and S = Slope of the calibration curve.

**Assay of acyclovir formulation**

Accurately weighted 20 tablets of Zovirax 400 and average powered wt equivalent to 10mg was transferred to 10ml volumetric flask and dissolve in 0.5ml of 0.1N $H_2SO_4$and solution was neutralized by 0.5 ml of 0.1N NaOH and volume made to 10 ml with of distilled water to produce a concentration of 1000µg/ml. 0.1ml of stock solution was taken and then diluted upto 10 ml by using distilled water to produce a concentration of 10µg/ml which is used as standard stock solution. The absorbance of solution at 252.6nm measured for estimation of acyclovir from tablet formulation. The analysis procedure was repeated six times with marketed formulation.

## RESULTS AND DISCUSSION

The absorbance maxima (λmax) of 10µg/ml standard solution of acyclovir was found to be 252.6nm (Figure 2). The linearity of the method was studied by taking range of solution from 2-10µg/ml. Calibration curve of absorbance vs concentration plotted. The drug obey Beers law with $R^2$ = 0.997(Figure 3 and 4). The method was precise as % R.S.D. was found to be 1.35%. The % recovery was found in the range of 99.90 to 101.2% which indicated accuracy of method (Table 1).The method was found to be robust as there was no change in absorbance of drug through small change in absorbance (Table 2). LOD and LOQ were found to be 2.3µg/ml and 7.0µg/ml respectively. The developed method was applicable for estimation of acyclovir in formulation the % recovery was found 103.2% (Table 3). The aim of the work was to perform the stress degradation studies on the Acyclovir using the UV Spectrophotometric method. The acyclovir was subjected to various stress degradation conditions, acidic, oxidative, photolytic and dry heat conditions. The overlay spectra of standard drug solution and stress degraded sample solution indicated the change in wavelength corresponding to maximum absorbance ($\lambda_{max}$) of standard drug solution 252.6nm, (Figure5), indicated that drug was unstable in alkaline and oxidative degradation

condition and stable in acid, thermal and photo degradation condition. Summary of method validation and stress degradation condition given in table 4 and 5.

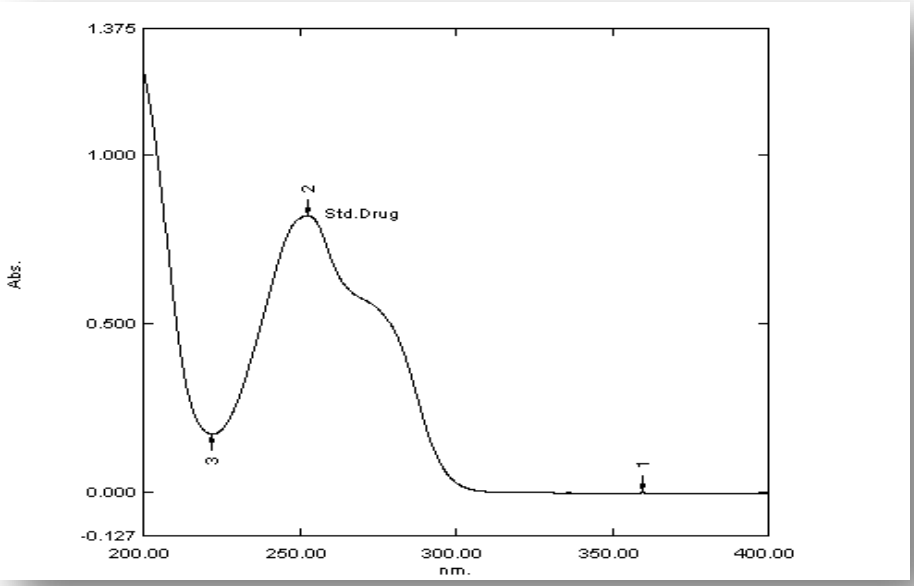



**Figure 2 Absorbance Spectra of Acyclovir showing at $\lambda_{max}$ 252.6nm.**

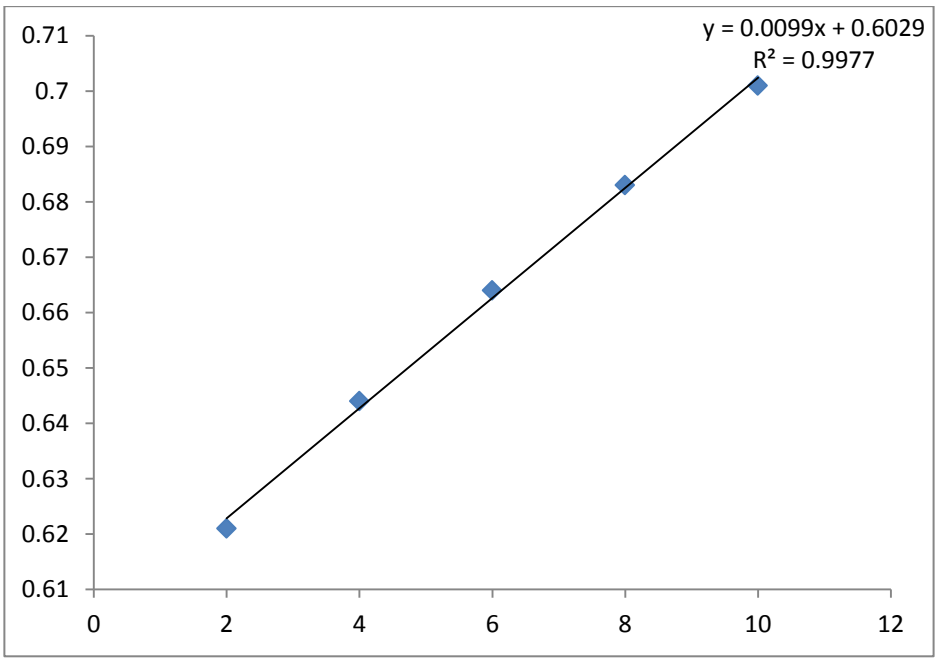



**Figure 3: Calibration curve of Acyclovir**

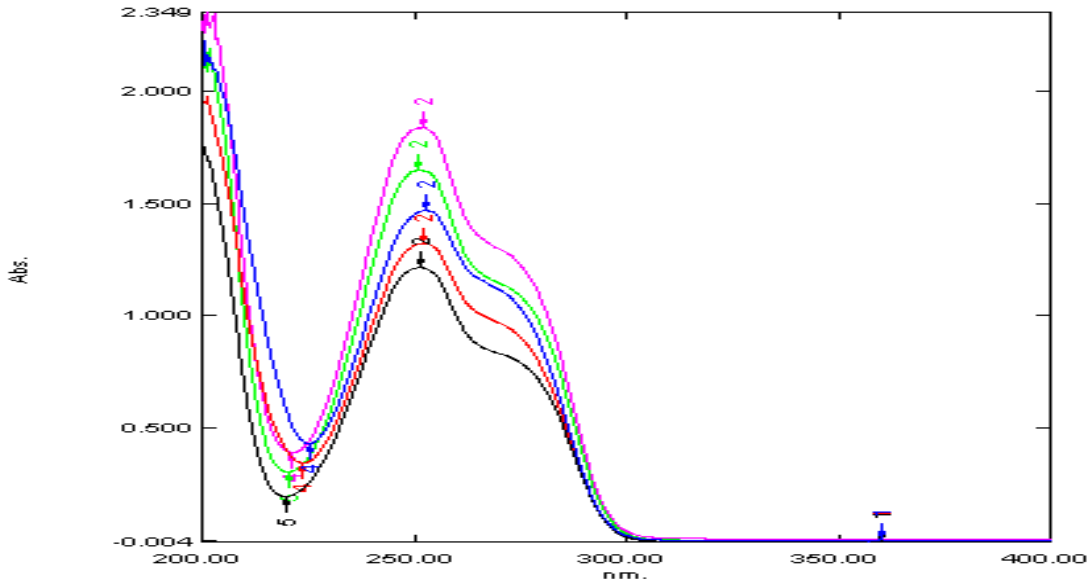
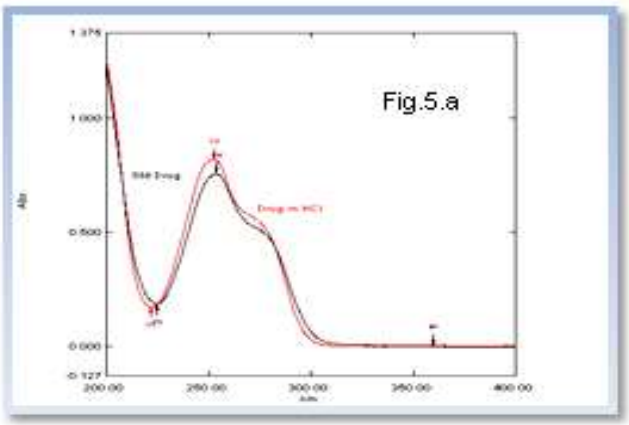
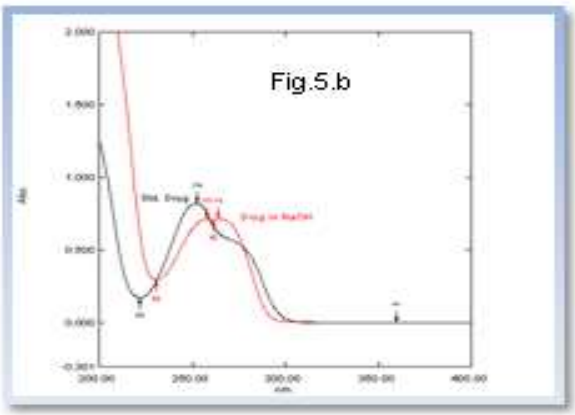



**Figure 4 Overlay Spectra of Acyclovir (Conc. 2 -10 µg/ml).**




**(a)**

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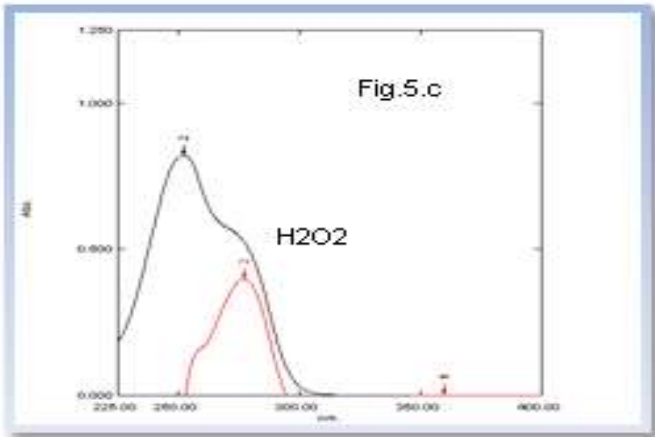


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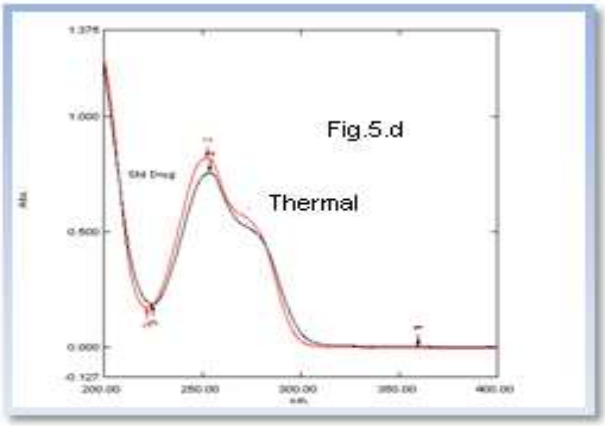


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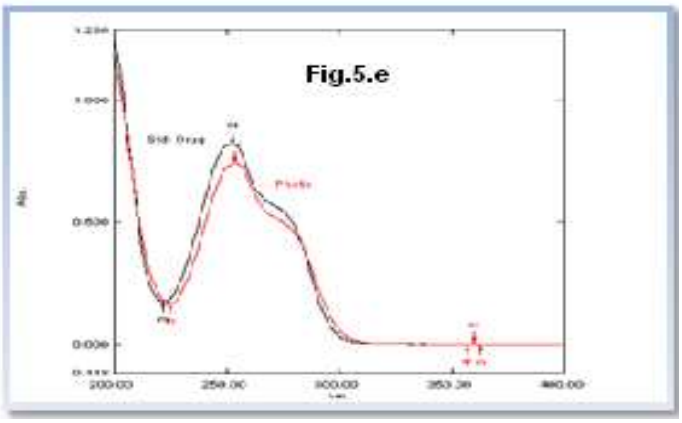


**(e)**

**Figure 5: Overlay Spectra of standard drug solution and stress degraded sample solution**

a. Acid Degraded Sample spectra-0.1N HCl at Room Temp.24hrs.

b. Base Degraded Sample spectra- 0.1N NaOH at Room Temp.2hr.

c. Hydrogen Peroxide- 3% H2O2; Room Temp., 1hr.

d. Thermal- Solid drug at $80^0$C in Oven, 24 hrs.

e. Photostability- 10µg/ml solution exposed to UV light, 24 hrs.

**Table 1: Accuracy of Acyclovir**

| Std. Conc.(µg/ml) | Sample. Conc.(µg/ml) | Total Conc.(µg/ml) | Mean % recovery | Statically analysis |
|---|---|---|---|---|
| 8 | 10 | 18 | 100.1 | SD = |
| 10 | 10 | 20 | 99.9 | 0.7%RSD = |
| 20 | 10 | 22 | 101.2 | 0.69 |

**Table 2: Result showing robustness of Acyclovir (n=3)**

| Parameter Changed | Conc.(µg/ml) | Abs. | Statically analysis |
|---|---|---|---|
| The scanning speed High | 10 | 1.356 | Mean= 1.305 |
| wavelength range was 200-350nm | 10 | 1.254 | SD = 01.15 %RSD = 1.15 |

**Table 3: Assay of Acyclovir Formulations (n = 6)**

| Marketed formulations | Sample. Conc.(µg/ml) | Mean absorbance | Mean % recovery | Statically analysis |
|---|---|---|---|---|
| Zovirax 400 | 10 | 1.354 | 103.2 | SD = 0.86 %RSD = 0.83 |

**Table 4: Summary of validation**

| Parameters | Result |
|---|---|
| Linearity indicated by correlation coefficient | $r^2$= 0.997. |
| Linear regression equation | Y = 0.009x + 0.602 |
| Precision indicated by % RSD | % RSD = 1.35 |
| Accuracy indicated by % recovery | % recovery = 99.90 to 101.2%. |
| LOD | 2.3µg/ml |
| LOQ | 7.0 µg/ml |
| Robustness indicated by % RSD | % RSD = 1.15 |
| Assay indicated by % recovery | % recovery = 98.5 to 103.2% |

**Table 5: Result of Stress Degradation Conditions for Acyclovir**

| Stress type | Conditions | Time | Change in Wavelength |
|---|---|---|---|
| Acid hydrolysis | 10µg/ml in 0.1N HCl at Room Temp. | 24 hours | No change in Wavelength |
| Base hydrolysis | 10µg/ml in 0.1NNaOH at Room Temp. | 2 hour | Wavelength change from 252.6 nm to 264.20 and 257.80nm. |
| Oxidative solution | 10µg/ml in 3% H2O2; Room Temp. | 1 hour | Wavelength change from 252.6 nm to 277.20. |
| Thermal | Solid drug at $80^0$C in Oven | 24 hours | No change in Wavelength |
| Photo-degradation | 10µg/ml solution exposed to UV light | 24 hours | No change in Wavelength |

## CONCLUSION

The developed stability indicating UV-Visible spectrophotometric method was found to be simple and cost effective. The developed UV Spectrophotometric method was found to be precise, specific and sensitive but disadvantage of the UV- Visible Spectrophotometric method is that it can not resolve standard drug from degradent. The stress degradation studies shown that Acyclovir undergoes degradation in alkaline, oxidative condition whereas it was stable when exposed to acidic, dry heat and photolytic conditions. The overlay spectra of standard drug solution and stress degraded sample solution indicated the change in wavelength corresponding to maximum absorbance ($\lambda_{max}$) to standard drug solution. The method is applicable for routine quality control analysis of drugs in combined dosage form with change in time and temperature.

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