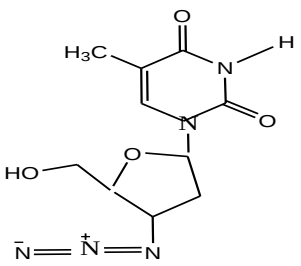
CHAPTER – IV

VISIBLE SPECTROPHOTOMETRIC AND RP-HPLC METHODS FOR THE DETERMINATION OF ZUDOVIDINE IN BULK AND DOSAGE FORMS

PART–A: NEW SENSITIVE VISIBLE SPECTROPHOTOMETRIC METHODS FORTHE DETERMINATION OF ZIDOVUDINE IN BULK AND DOSAGE FORMS

4.01-A: DRUG PROFILE

Zidovudine (ZDV) **is chemically known as** 1-[(2*R*,4*S*,5*S*)-4-azido-5-(hydroxymethyl)oxolan-2-yl]-5-methyl-1,2,3,4-tetrahydropyrimidine-2,4-dione (also called ZDV) is a nucleoside analog reverse transcriptase inhibitor (NRTI), a type of antiretroviral drug used for the treatment of acquired immunodeficiency syndrome. It is an analog of thymidine[1,2].It is formulated marketed in dosage forms by GlaxoSmithKline in the brand name **Retrovir** [label claim 150 mg or 300 mg tablets].



Zidovudine

Literature survey reveals many high performance liquid chromatographic methods have been reported for its estimation mostlyin body fluids such as human serum[3-6], human plasma[7-12], human plasma and urine[13] and rat plasma [14] and only one HPLC method has been reported for the determination of Zidovudine in pure drug and marketed tablets. An UV-spectrophotometric method has also been reported for the simultaneous determination of Zidovudine and lamivudine in human serum [16] and in pharmaceuticals [17,18]. Only one visible spectrophotometric method [19] has been reported for the determination of ZDV in pure form and in dosage forms. However, the analytically useful functional groups present in Zidovudine have not been fully exploited and therefore still offers a scope to develop new visible spectrophotometric methods with better sensitivity, selectivity, precision and accuracy.

The author has made some attempts in this direction and succeeded in developing eight sensitive and cost-effective new visible spectrophotometric methods for the determination of Zidovudine in pure and in dosage forms. The proposed methods developed by the author had the advantages of speed and simplicity besides being accurate and precise, and can be adopted by the pharmaceutical laboratories for routine quality control analysis.

4.02-A: EXPERIMENTAL

i) UV-Visible Spectrophotometer: An Elico SL-159 model,2nm high resolution, double beam, 1cm length quartz coated optics; Wavelength range190-1100nm ; High stability, linearity, precision instrument was used for all the spectral measurements.

ii) Preparation of reagents: Analytical grade chemicals and reagents were used in the preparation of solutions and all the solutions were prepared in triply distilled water.

Method M_{1a}: Solutions of various reagents such as **MBTH solution** (Fluka; 0.2%, 8.56×10^{-3}M), **IBDA solution**(BDH; 0.2%, 9.35×10^{-3}M)and **Acetic acid solution** (Qualigens; 2.3M) were prepared in the same way as described under SVD in chapter – II.

Method M_{1c}: Solutions of various reagents such as **MBTH solution** (Fluka; 0.2%, 8.56×10^{-3}M), **$NaIO_4$ solution**(BDH; 0.2%, 9.35×10^{-3}M)and **Acetic acid solution** (Qualigens; 2.3M) were prepared in the same way as described under SVD in chapter – II.

Method M_3: Solution of various reagents such as **Brucine Solution** (Loba; 0.2%, 5.067×10^{-3}M) and **$NaIO_4$ solution,**(BDH; 0.2%, 9.35×10^{-3}M) were prepared in the same way as described under SVD in chapter – II.**Conc.H_2SO_4** (Qualigens,2.3M) is used as it is.

Method – M_7

Isatin solution (Sd Fine,0.04%, 2.718 x 10^{-2}M)	:	Prepared by dissolving 40mg of Isatin in 100mL of CH_3COOH.
H_2SO_4 (Qualigens)	:	Used as it is.

Method – M_8

Vanillin solution (BDH,0.4%, 2.63 x 10^{-3}M)	:	Prepared by dissolving 400mg of Vanillin in 100mL of CH_3OH.
H_2SO_4 (Merck,Conc.)	:	Used as it is.

Method M_{10}: Solution of various reagents such as chloranilic acid solution, p-CA solution (Sd-fine; 0.1%, 4.785x10^{-3}M) were prepared in the same way as described under SVD in chapter – II.

Methods M_{11a} and **M_{11b}:** Solutions of various reagents such as **TPooo solution** (Fluka; 0.2%, 5.70x10^{-3}M) and **ARS solution** (0.2%, 5.84x10^{-3}M) and HCl (Qualigens; 0.1M) were prepared in the same way as described under in **Chapter – II.** Chloroform of analytical grade is used as it is.

iii) Preparation of standard drug solution

0.1% stock solution of zidovudine was freshly prepared by transferring accurately weighed 100mg of zidovudine into 100mL volumetric flask and dissolved in double distilled water, and then made up to the mark. Then working standard solutions, 100μg mL^{-1} and 200μg mL^{-1} are prepared by transferring 10.0mL and 20.0mL of the stock solution into two

100mL standard flasks respectively and made up to the mark. 100μg mL^{-1} working standard solution was used for the following methods **M_{11a}** and **M_{11b}**. , 200μg mL^{-1} working standard solution was used for the following methods **M_{1a}**, **M_{1c}, M_3, M_7, M_8,** and **M_{10}**.

iv) Pharmaceutical preparations

Twenty tablets of each zidovudine drug was finely powdered in a small dish. Fifty mg of this powder was dissolved in about 10.0mL of ethanol and filtered through a Whatman No. 42 filter paper. The filtrate was made up to mark with distilled water in a 100mL volumetric flask. A suitable volume of the filtrate was accurately diluted with water to get a sample concentration of 100μg.mL^{-1} for the methods **M_{11a}** and **M_{11b}** and 200μg.mL^{-1} working standard solution was used for the following methods **M_{1a}**, **M_{1c}, M_3, M_7, M_8** and **M_{10}.** An aliquot of this solution was used for the determination of each zidovudine drug as per the procedures described earlier.

4.03-A: PROPOSED PROCEDURES

Method M_{1a}

Aliquots of working standard solution of zidovudine (0.5-3.0mL, 200μg.mL^{-1}) were transferred into a series of 10.0mL calibrated tubes. Then 1.0mL (8.56 x 10^{-3}M) of MBTH solution and 2.0mL of IBDA solution were added and kept aside for 5min. Then the total volume was made up to the mark with distilled water. The absorbance was measured at 590nm against a similar reagent blank. The amount of zidovudine was computed from its calibration graph. (**Fig.4.09,P,167).**

Method M_{1c}

In to a series of six 10 mLcalibrated tubes different aliquots of standard zidovudine solution (0.5-3.0mL,200μg.mL^{-1}) were transferred. Then 1.0mL (9.35 x 10^{-3}M) of $NaIO_4$ solution,1.0mL of acetic acid solution were and the total volume was adjusted to 10.0 mL and kept in a water bath for 45min. The solutions were cooled suddenly. After that 1.0mL (8.56 x 10^{-3}M) of MBTH solution was added and kept aside for 10min. The volume was made up to the mark with distilled water. The absorbance was measured at 650nm against a similar reagent blank. The amount of zidovudine was computed from its calibration graph. (**Fig.4.10, P…167)**.

Method M_3

Different aliquots of zidovudine working standard solution (0.5mL-3.0mL,200μg.mL^{-1}) were transferred into different 25mL graduated tubes.2.0mL of 0.2% (5.067 x 10^{-3}M) of brucine solution, 2.0mL (9.35x10^{-3}M)of sodium metaperiodate solution and 2.0mL (2.3M) of sulphuric acid were added to each tube and the total volume was made upto 9mL with distilled water. The tubes were thoroughly shaken and placed in a boiling water bath for 15min. The reaction mixture was then cooled to room temperature and total volume was adjusted to 25mL with distilled water. The absorbance of each solution was measured at 530nm against a reagent blank. The amount of zidovudine present in the sample was computed from the calibration graph (**Fig.4.11, P167**).

Method M_7[Isatin]

Aliquots of zidovudine solution (0.5 - 3.0mL,200μg.mL^{-1}) were transferred into different 10mL graduated tubes and the volume of the each test tube is adjusted to 3.0mL with methanol. To each of these tubes 1.0mL of isatin (2.718 x 10^{-3}M) and 1.0mL of sulphuric acid

were added and the tubes were thoroughly shaken. The reaction mixture was then cooled to room temperature and total volume was adjusted to 10mL with methanol. The absorbance of each solution was measured at 640nm against a reagent blank. The amount of zidovudine present in the sample was computed from the calibration graph (**Fig.4.12, P.167)**.

Method M_8[Vanillin]

To each of 25mL calibrated tubes, aliquots (0.5 - 2.5mL, 200μg mL^{-1}) of standard zidovudine solution, 2.0mL of vanillin and 3.0mL of con sulphuric acid were added successively and the total volume in each flask was brought to 20mL by the addition of methanol and placed in heating water bath (maintained at 50^0C) for 15min. Then the flasks were colored and made upto the mark with methanol and the absorbances were measured at 540nm against a reagent blank prepared in a similar way. The con of drug in a sample was computed from Beer-Lambert plot (**Fig.4.13, P.168**).

Method M_{10}

Into a series of 20.0mLcalibrated tubes containing aliquots of working standard solution of zidovudine (0.5mL-3.0mL, 200μg.mL^{-1}), 2.0mL of p-chloranilic acid (4.785 x 10^{-3}M) was added and kept aside for 30 min at lab temperature. The volume in each tube was made up to the mark with chloroform. The absorbance of the colored species was measured at 540nm against a reagent blank. The amount of the drug was calculated from Beer's law plot (**Fig.4.14, P.168**).

Method M_{11a} &M_{11b}

Aliquots of zidovudine solution (0.5 - 3.0mL,200μg.mL^{-1}) were transferred into 125mL separating funnels containing aliquots of standard zidovudine solution (0.5-3.0mL,

100μg.mL^{-1} for the methods M_{11a} and M_{11b} respectively), 6.0mL of 0.1M HCI solution, 2.0mL 0.2% TPooo dye solution $M_{11a}$and 1.0mL of 0.2% ARS dye solution M_{11b} successfully. The total volume of aqueous phase in each separating funnel was adjusted to 15mL with distilled water. To each separating funnel 10.0mL of chloroform was added and the contents were shaken for 2min. The two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at λmax (650nm, ARS and 430nm, TPooo) against a similar reagent blank. The amount of zidovudine was deduced from the calibration curve ARS and TPooo (**Fig.4.15& 4.16, P.168**).

4.04-A: RESULTS AND DISCUSSIONS

i. Spectral Characteristics: In order to ascertain the optimum wavelength of maximum absorption (λ_{max}) of the colored species formed in the above methods, specified amounts of zidovudine were taken and colors were developed separately by following the above procedures. The absorption spectra were scanned on a spectrophotometer in the wave length region of 340 to 900nm against similar reagent blank or distilled water. The reagent blank absorption spectrum of each method was also recorded against distilled water. The results were graphically represented in **Fig. 4.01 to 4.08, P.165-166**. The absorption curves of the colored species in each method show characteristics absorption maxima where as the blank in each method has low or no absorption in this region.

ii. Optimization of the proposed methods[Parameters fixation]: The optimum conditions for the color development of methods (**M_{1a}, M_{1c}, M_3, M_7, M_8, M_{10}, M_{11a}** and **M_{11b}**) were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species. The following experiments were conducted for this purpose and the conditions so obtained were incorporated in recommended procedures.**For Methods M_{1a}, M_{1c}, M_3, M_{10}, M_{11a}** and **M_{11b}.** The optimum conditions

established for methods **M_{1a}**, **M_{1c}**, **M_3**, **M_{10}**, **M_{11a}** and **M_{11b}** were found to be same as described in **Chapter II**.

For Method M_7 [Isatin]: This method involves the condensation of the zidovudine with Isatin in the presence of acid. The effect of various parameters, such as concentration and volume of Isatin, nature and strength of acid, order of addition of reagents, solvent for final dilution were studied and the optimum conditions developed and actual conditions chosen for the procedure are reported in **Table.4.01, P.171**.

For Method M_8 [Vanillin]: This method involves the condensation of the zidovudine with Vanillin in the presence of acid. The effect of various parameters, such as concentration and volume of Vanillin, nature and strength of acid, order of addition of reagents, solvent for final dilution were studied and the optimum conditions and actual conditions chosen for the procedure are recorded in **Table.4.02, P.172.**

iii) Optical Characteristics

In order to test whether the colored species formed in the above methods, adhere to Beer's law the absorbance's at appropriate wave lengths of a set of solutions containing varying amounts of zidovudine and specified amounts of reagents (as given in the proposed procedures for each method) were recorded against the corresponding reagent blanks. The Beer's law plots of these systems are recorded against the corresponding reagent blanks are recorded graphically (**Figs.4.09** to **4.16, P.167-168**). The optimum photometric range (Ringbom plot) of these systems is recorded graphically (**Figs.4.17** to **4.24**, **P.**1**69,170**). A linear correlation was found between the absorbance and the concentration of zidovudine for all the proposed methods respectively.

iv) Linearity Range and Analytical data

Linearity ranges for each proposed spectrophotometric method for quantitative analysis of zidovudine, were made by plotting calibration curves over the concentration ranges cited. The statistical parameters (optical characteristics) such as Beer's law limits, Sandell's sensitivity, molar absorptivity, percent relative standard deviation (calculated from six replicate samples containing 3/4th of the amount of the upper beer's law limits) were calculated for all the proposed methods and the results are summarized in **Table.4.05a & 4.05b,P.175-176.** Correlation coefficient values, suggesting a perfect linearity between the absorbance and concentration of drugs in the Beer's law limits were studied. Regression characteristics like standard deviation of slope (Sb), standard deviation of intercept (Sa), standard error of estimation (Se) and detection limit were calculated for all the methods and are shown in **Table.4.05a & 4.05b,P.176.**

v) Sensitivity [Detection and quantification limits]

In accordance with the formula given by International Conference on Harmonization (ICH), LOD is defined as 3 S_a/b and LOQ is defined as 10S_a/b, where S_a is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and b the slope of the calibration curve. The values are also summarized in **Table.4.05a & 4.05b,P.176-5-176.**

vi) Precision

Precision refers to the reproducibility of measurements within a set of measurements. One of the most common statistical terms employed is the standard deviation. The precision of each proposed method was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of zidovudine in total solution. The percent

relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods (**Table.4.03, P.173**).

vii) Accuracy

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet powder was spiked with pure zidovudine at one concentration levels and the total was found by the proposed methods. In all cases, the added zidovudinerecovery percentage values ranged between 96.58 and 104.3 %. The results of this study given in **Table.4.04,P.174,** indicated that the recovery was good, and that the co formulated substances did not interfere in the determination.

viii)Application to tablets analysis

The proposed methods were applied to determine zidovudine in two brands of tablets and the results are summarized in **Table.4.06a & 4.06b,P.177-178.** The results were compared with those of the reference method which consisted of the measurement of absorbance of the tablet extract at 240nm in methanolic medium. Statistical analysis of the results using Student's t-test for accuracy and F-test for precision revealed no significant difference between the proposed methods and the literature method at the 95% confidence level with respect to accuracy and precision.

ix)Color reactions of the proposed methods

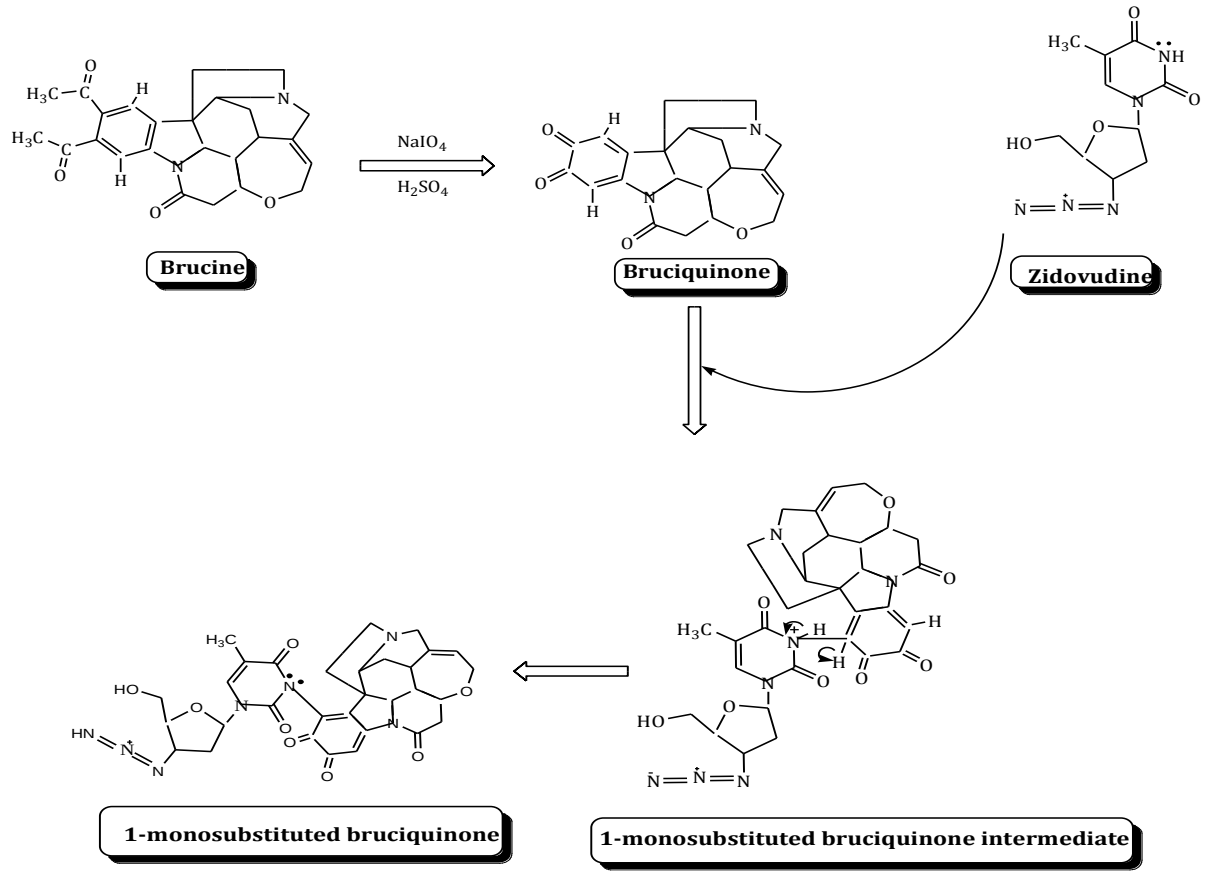
The reviews concerning the reagents used for the development of color by exploiting appropriate functional moieties in ZDV, an attempt has been made to indicate the nature of colored species in each of the proposed methods.

Methods –M_{1a} & M_{1c}

Under the optimized reaction conditions MBTH looses two electrons and one proton during oxidation with oxidants such as IBDA and $NaIO_4$ forming an electrophilic intermediate, which is the active coupling species. These active species reacts with the coupler (i.e.) Zidovudine by electrophilic attack on the most nucleophilic site in the benzene ring of the coupler giving oxidative coupled products.

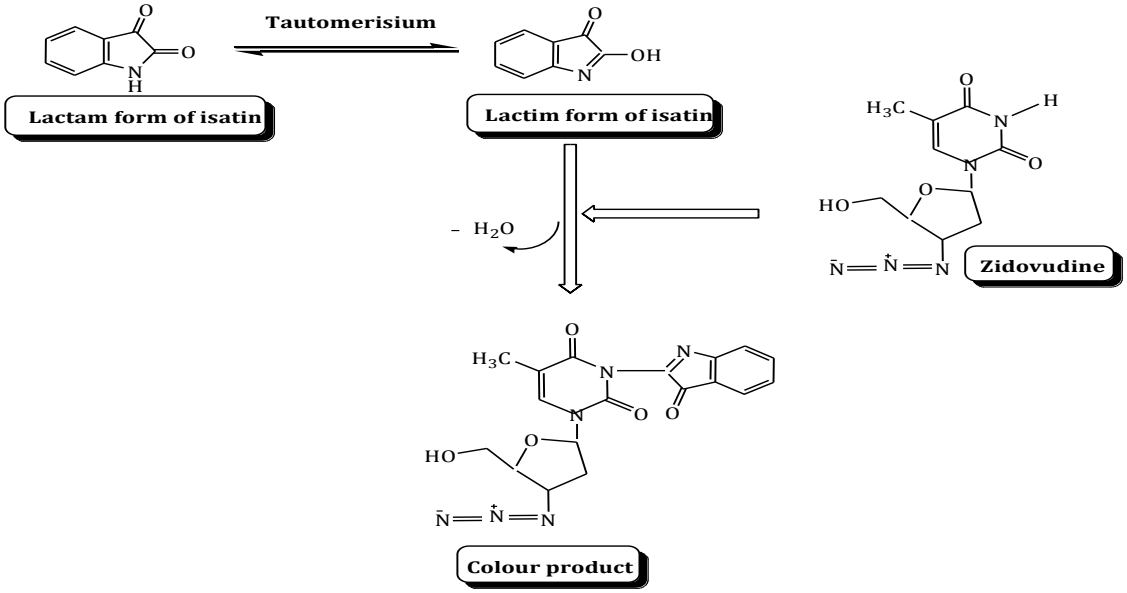
Method-M_3

The dimethoxy benzene nucleus of brucine is attacked by IO_4^- with the formation of o-quinine(bruciquinone),which in turn undergoes nucleophilic attack on the most electron rich portion of the coupler (secondary nitrogen), to give 1-monosubstituted bruciquinone derivative. The reaction of **ZDV** with brucine in the presence of IO_4^- is described in scheme.



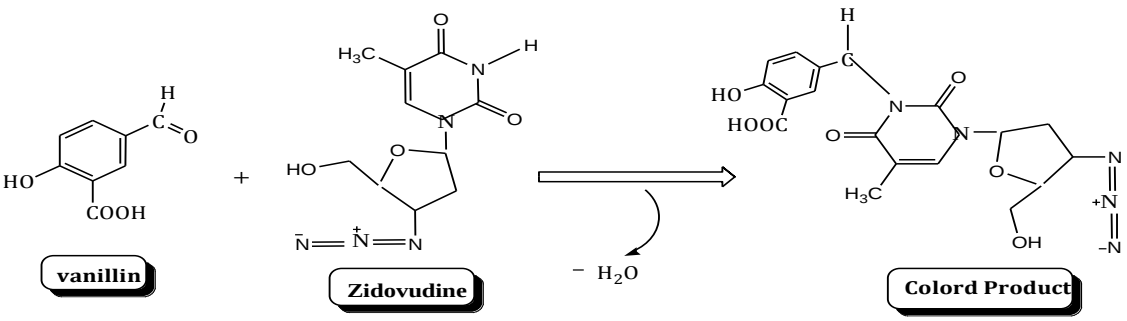
Method – M_7

The secondary amino group of the drug (zidovudine) reacts with the keto group of the isatin resulting in the formation of colored species. The colored species formation may be represented as given below.



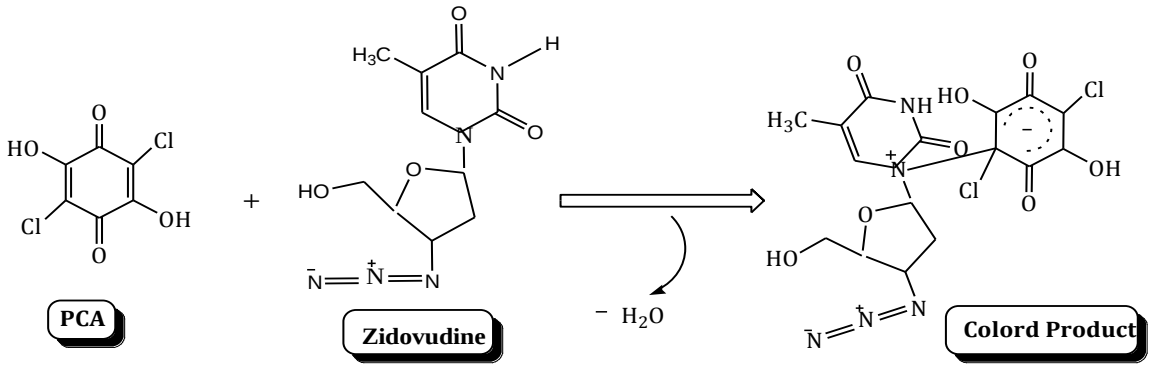
Method – M_8

The secondary amino group of the zidovudine reacts with the keto group of the vanillin resulting in the formation of colored species. The colored species formation may be represented as given below.



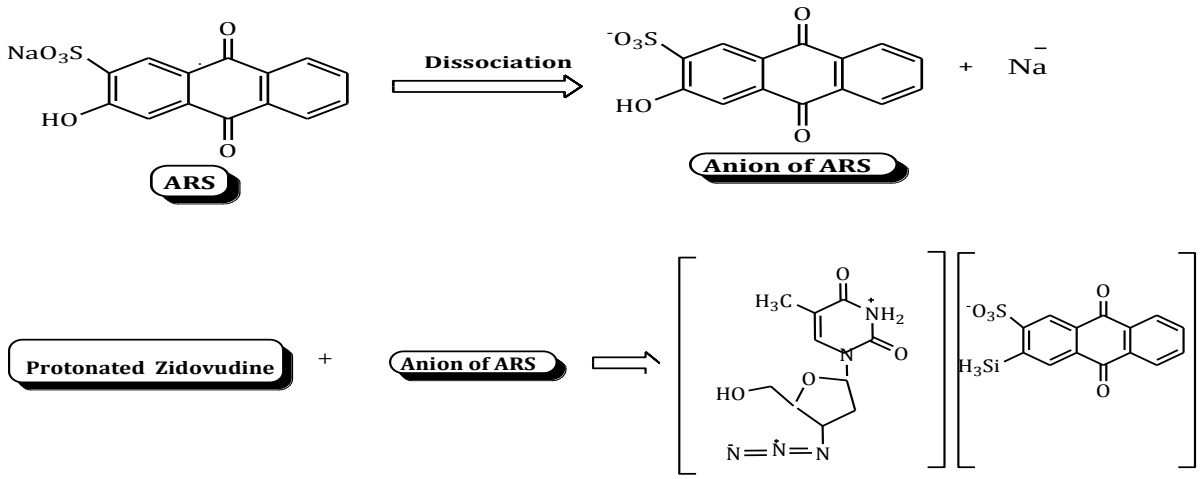
Method –M_{10}

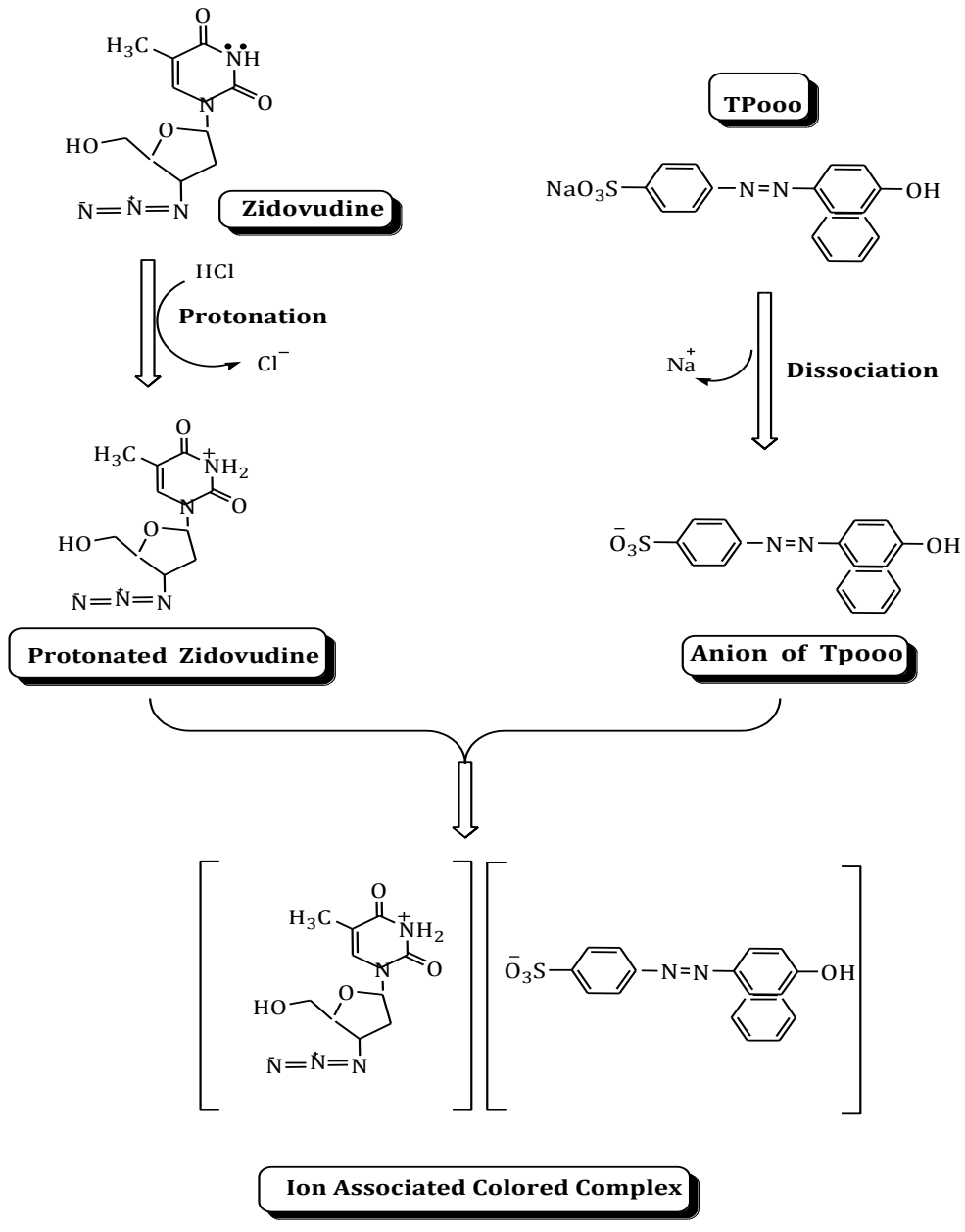
As the drug possesses tertiary amine group, it functions as an electron donor and participate in charge transfer interaction with p-CA which is known as electron acceptor. The color species formation appears as electron acceptor. The color species formation appears to be due to the formation of radical ion.



Method –M_{11a} & M_{11b}

The selected drug (Zidovudine) possessing a primary amine group forms 1:1 ion-ion association complex with two acid dyes such as TPooo (M_{11a}) and ARS (M_{11b}) Which is extractable into chloroform from the aqueous phase. When the drug is treated with hydrochloric acid, protonation takes place on tertiary nitrogen atom. The protonated nitrogen having positive charge is associated with anion of the acidic dyes and behaves as a single unit being held together by electrostatic force of attraction.


Dissociation
ARS
Anion of ARS
Protonated Zidovudine
Anion of ARS


Zidovudine
HCl
Protonation
TPooo
Dissociation
Protonated Zidovudine
Anion of Tpooo
Ion Associated Colored Complex

4.06-A: CONCLUSIONS

The proposed spectrophotometric methods for the determination of Zidovudine are simple, accurate, precise and cheap. The statistical analyses show that the data from the proposed method are in good agreement with those of the reported method. Thus it can be extended for routine analysis of Zidovudine in pharmaceutical industries and research laboratories.

Fig. 4.01. Absorption spectrum of ZDV-MBTH,IBDA(M_{1a})

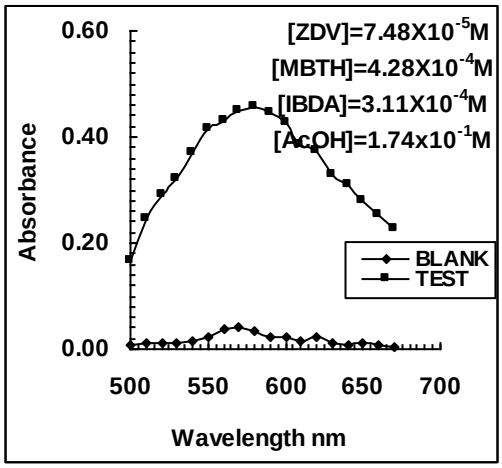


Fig.4.02. Absorption spectrum of ZDV-MBTH, $NaIO_4$(M_2)

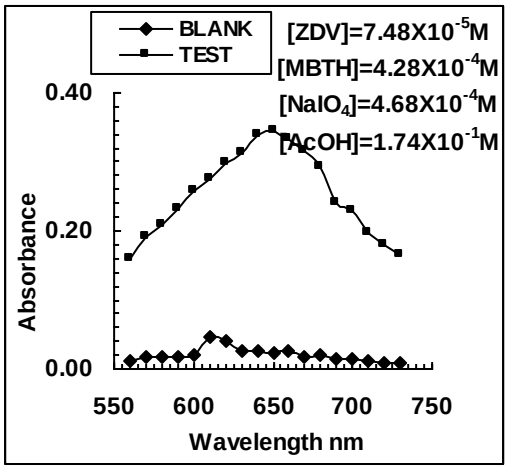


Fig. 4.03. Absorption spectrum of ZDV-Brucine,$NaIO_4$(M_3)

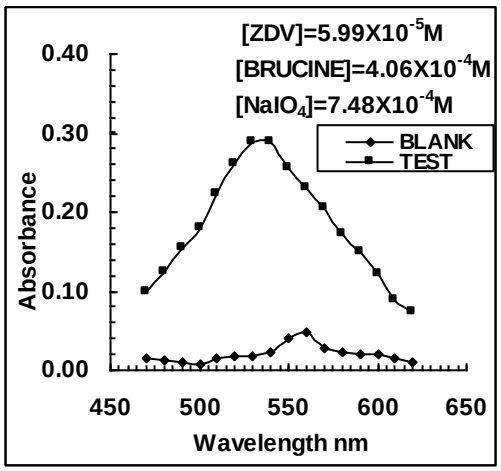


Fig. 4.04. Absorption spectrum of ZDV-Isatin(M_7)

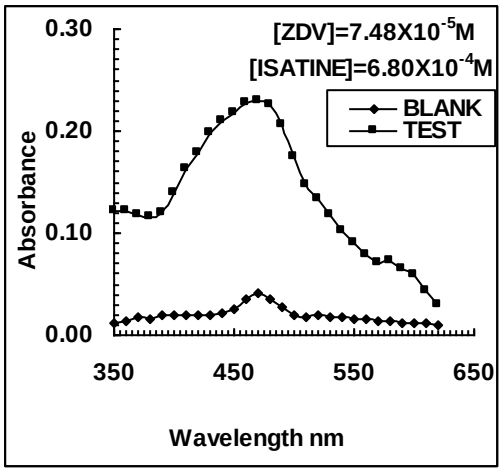


Fig.4.05. Absorption spectrum of ZDV-Vanillin (M_8)

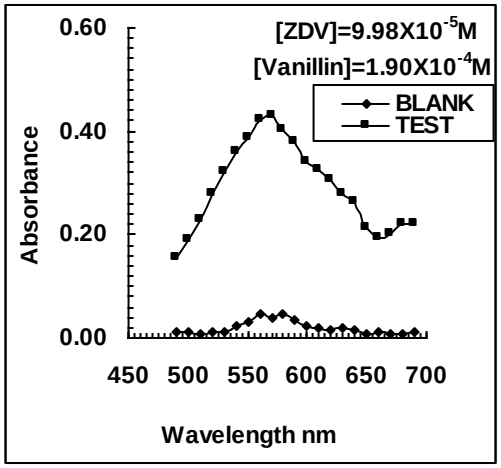


Fig.4.06.Absorption spectrum of ZDV-P-CA (M_{10})

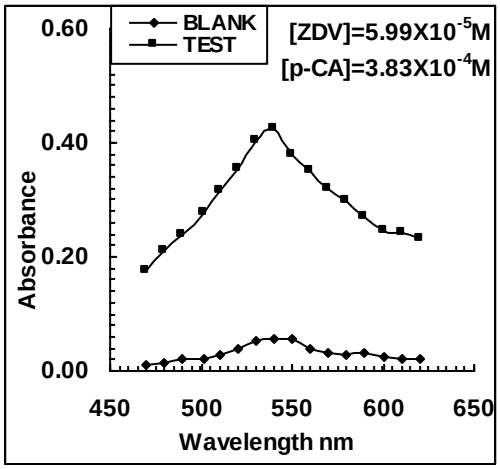


Fig. 4.07. Absorption spectrum of ZDV-TPooo,$CHCl_3$(M_{11a})

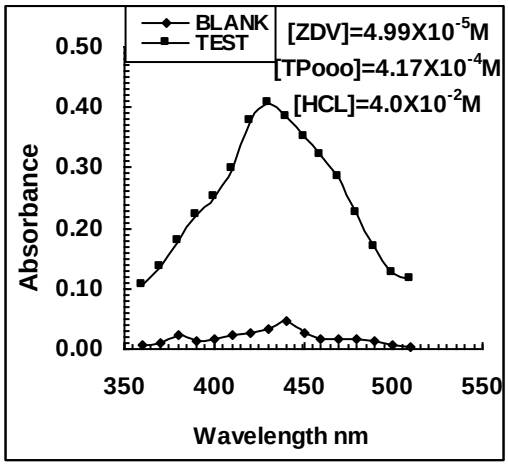


Fig.4.08. Absorption spectrum of ZDV-ARS,$CHCl_3$ (M_{11b})

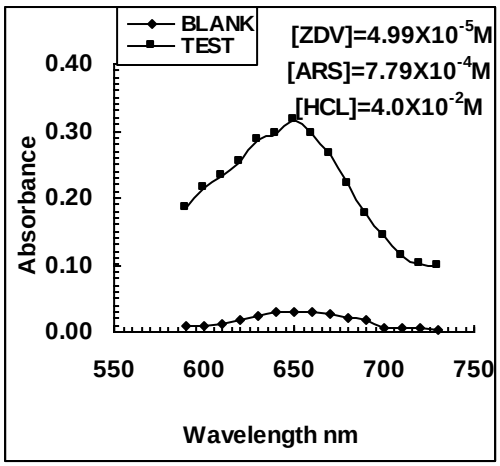


Fig. 4.09: Beer's law plot of ZDV with with MBTH, IBDA,AcOH (M_{1a})

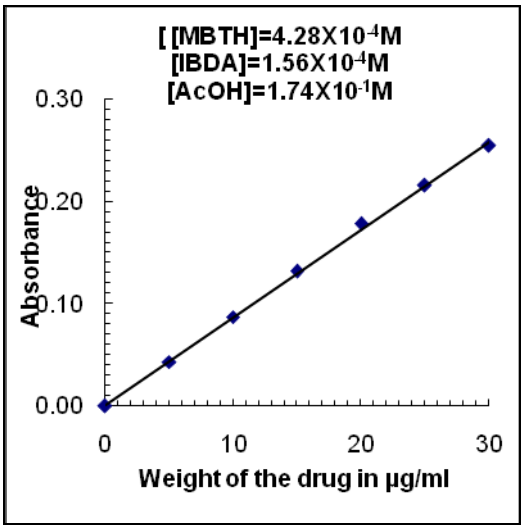


Fig. 4.10: Beer's law plot of ZDV MBTH – $NaIO_4$, AcOH(M_{1c})

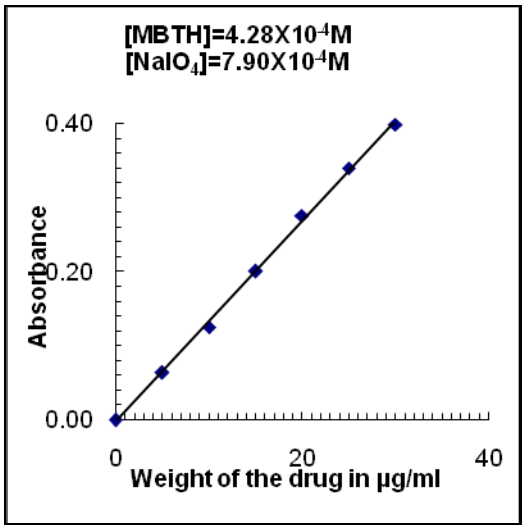


Fig. 4.11. Beer's law plot of ZDV-Brucine,$NaIO_4$(M_3)

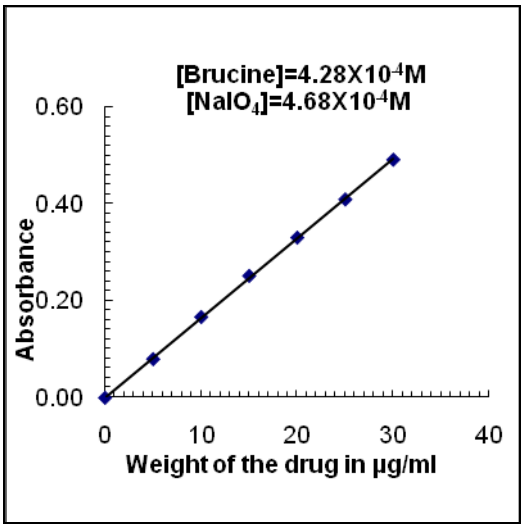


Fig. 4.12. Beer's law plot of ZDV-Isatin(M_7)

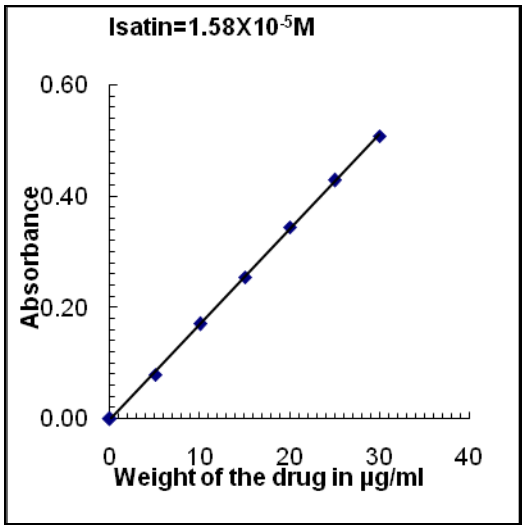


Fig. 4.13. Beer's law plot of ZDV with Vanillin(M_8)

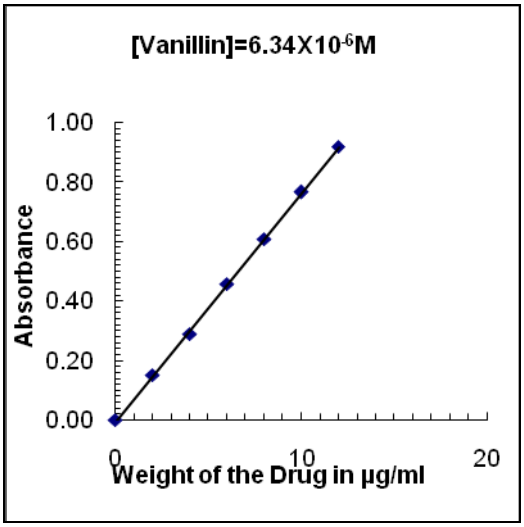


Fig.4.14. Beer's law plot of of ZDV with P-CA(M_{10})

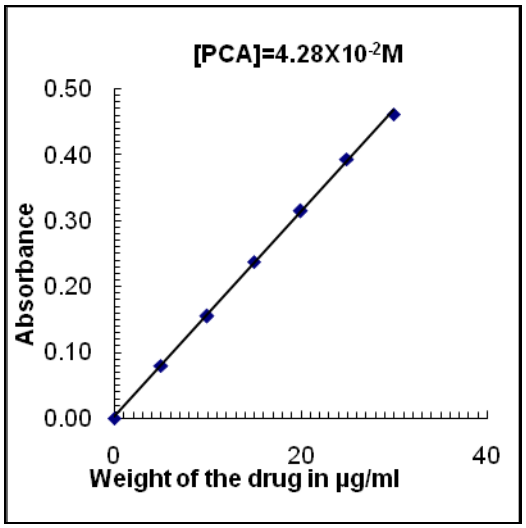


Fig. 4.15. Beer's law plot of of ZDV with TPooo(M_{11a})

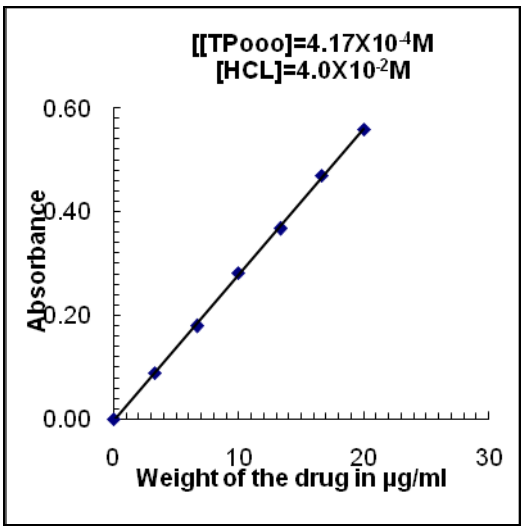


Fig. 4.16. Beer's law plot of of ZDV with ARS(M_{11b})

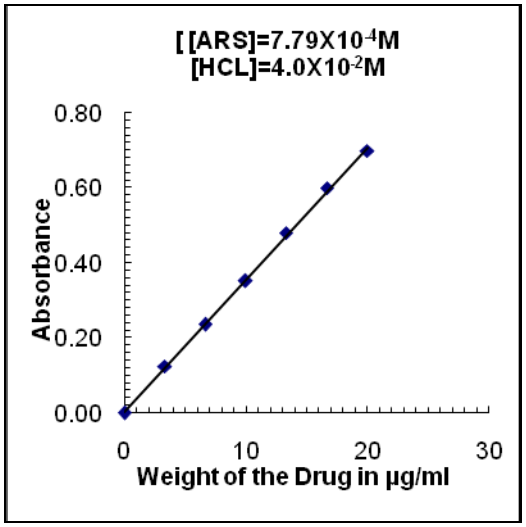


Fig. 4.17: Ringbom plot of ZDV with MBTH – IBDA(M_{1a})

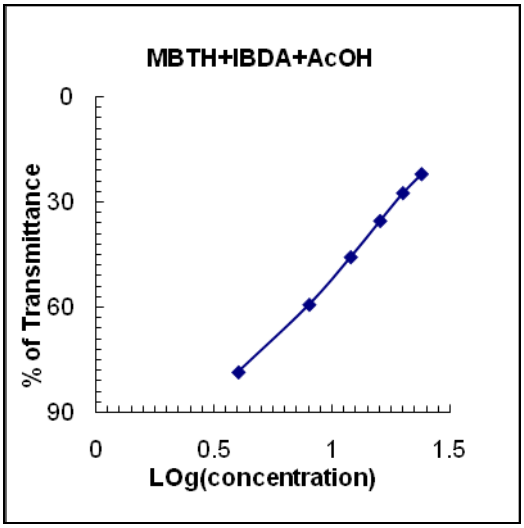


Fig. 4.18: Ringbom plot of ZDV with MBTH – $NaIO_4$(M_{1c})

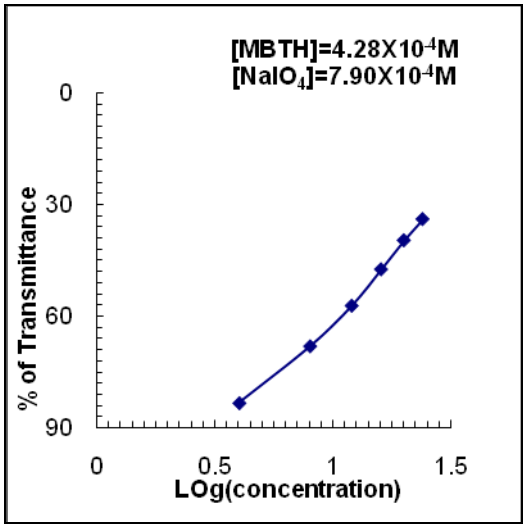


Fig.4.19: Ringbom plot of ZDV with BRUCINE – $NaIO_4$(M_3)

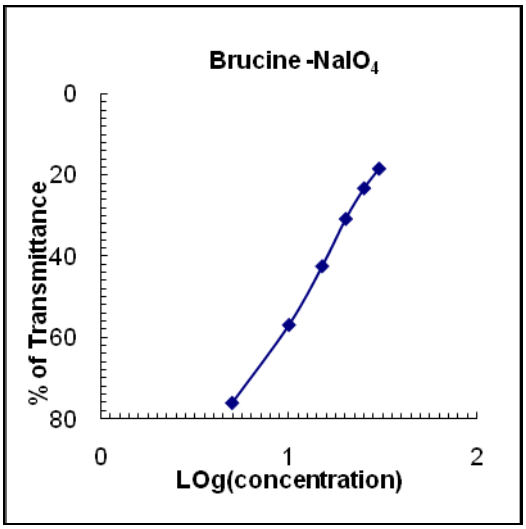


Fig. 4.20: Ringbom plot of ZDV with Isatin(M_7)

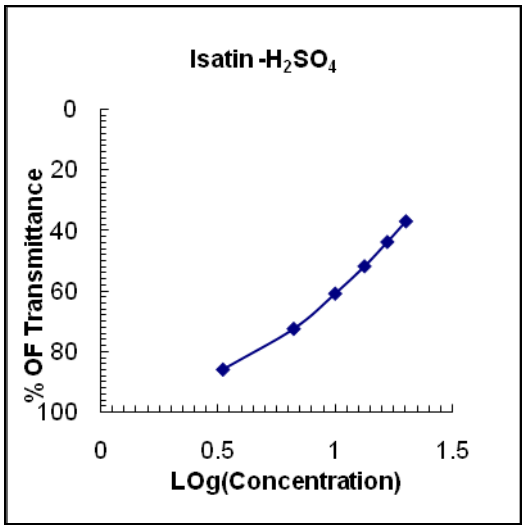


Fig. 4.21: Ringbom plot of ZDV with Vanillin(M_8)

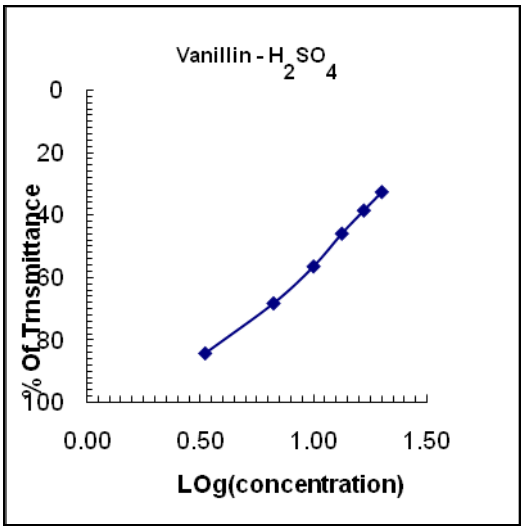


Fig. 4.22: Ringbom plot of ZDV with PCA(M_{10})

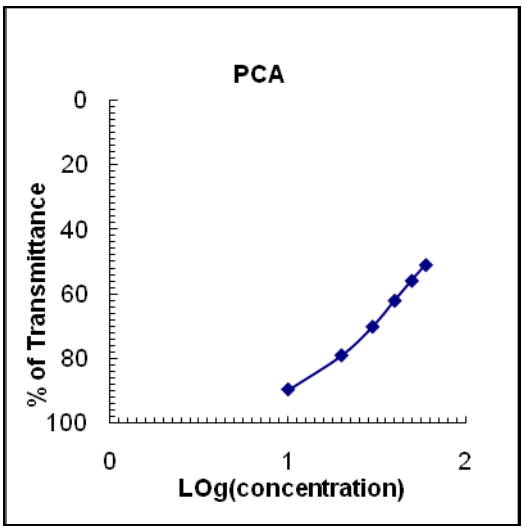


Fig. 4.23: Ringbom plot of ZDV with TPooo(M_{11a})

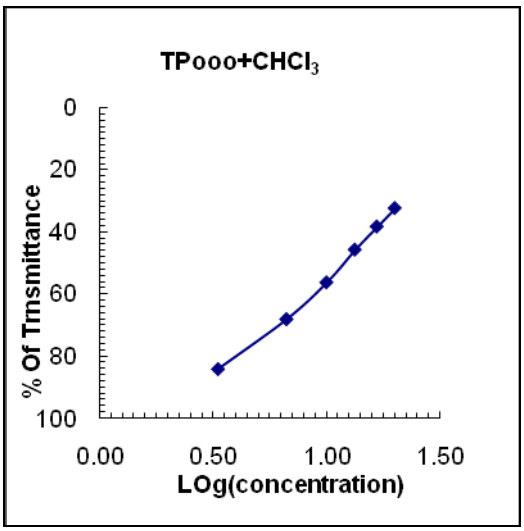


Fig. 4.24: Ringbom plot of ZDV with ARS(M_{11b})

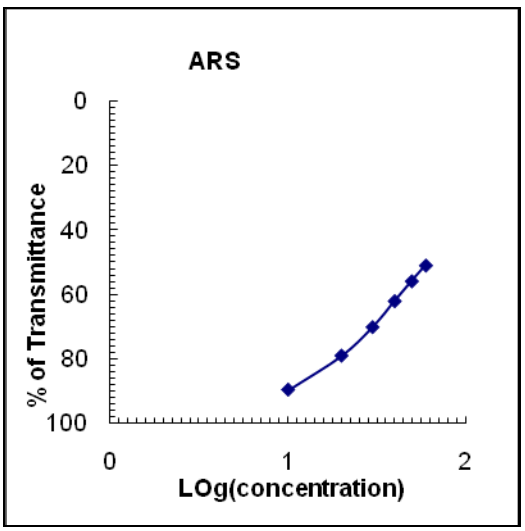


Table.4.01
Optimum conditions established in method M_7 for Zidovudine

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	630 - 670	640	
Volume of Isatin (2.718 x 10^{-2}M)	0.5 - 1.5mL	1.0mL	1.0mL of isatin (2.718 x 10^{-2}M) was necessary for covering broad range of Beer's law limits.
Effect of vol. Of con H_2SO_4 on color development	0.5 - 2.0mL	1.0mL	<1.0mL of con H_2SO_4 results in low absorbance values and >4.0mL results in instability of the colored product.
Effect of the order of addition reagents on color development.	ZDV, Isatin, Con. H_2SO_4	ZDV, Isatin, Con. H_2SO_4	If the order of addition is changed, low absorbance values resulted.
Effect of temperature and time	70 - 80^0C 10 - 20min	75^0C 15min.	The stability of the colored species was found to be less, if the temperature exceeds 80^0C.
Stability period after final dilution	Immediate - 30min	5min	--

Table.4.02
Optimum conditions established in method M_8 for Zidovudine

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	530-570	540	
Volume of Vanillin (2.63 x 10^{-3}M)	1.5 - 3.0mL	2.0mL	2.0mL of Vanillin (2.63 x 10^{-3}M) was necessary for covering broad range of Beer's law limits.
Effect of vol. of con H_2SO_4 on color development.	2.0 – 4.0mL	3.0mL	<1.5mL of con H_2SO_4 results in low absorbance values and >4.0mL results in instability of the colored product.
Effect of the order of addition reagents on color development.	ZDV, Vanillin, Con. H_2SO_4	ZDV, Vanillin, Con. H_2SO_4	If the order of addition is changed, low absorbance values resulted.
Effect of temperature and time	40 - 50^0C 10 - 20min	50^0C 15min.	Above 50^0C methanol evaporates.
Stability period after final dilution	Immediate - 30min	5min	--

Table.4.03
Intra-day precision and accuracy results

Proposed methods	ZDV taken µg.mL^{-1}	Intra-day[a]		
		ZDV found[c], µg.mL^{-1}	Precision[d]	Accuracy[e]
Method M_{1a}	20.0	19.89	0.272	-0.55
Method M_{1c}	20.0	19.85	0.402	-0.75
Method M_3	20.0	19.87	0.457	-0.65
Method M_7	20.0	19.95	0.309	-0.25
Method M_8	30.0	30.26	1.64	0.86
Method M_{10}	20.0	19.90	0.265	-0.50
Method M_{11a}	15.0	14.88	0.299	-0.8
Method M_{11b}	15.0	14.91	0.404	-0.60

a = 6 replicates;c= mean±standard error; d=relative standard deviation, %; e= bias %

Table.4.04

The results of percentage recovery values of Zidovudine by the proposed spectrophotometric methods

Proposed methods	SVD in tablet $\mu g.mL^{-1}$	Pure SVD added $\mu g.mL^{-1}$	Total found $\mu g.mL^{-1}$±SD	Pure ABS recovered ±%RSD*
Method M_{1a}	20.0	10.0	30.02± 0.42	100.06±1.39
Method M_{1c}	20.0	10.0	29.86± 0.56	99.53±1.87
Method M_3	20.0	10.0	30.07± 0.49	100.02±1.63
Method M_7	20.0	10.0	29.80± 0.33	99.33±1.10
Method M_8	30.0	10.0	39.92± 0.18	99.80±0.45
Method M_{10}	20.0	10.0	29.96± 0.54	99.86±1.80
Method M_{11a}	15.0	10.0	24.88± 0.21	99.52±0.844
Method M_{11b}	15.0	10.0	24.84± 0.40	99.36±1.65

Table.4.05a

Optical and regression characteristics of the proposed methods for Zidovudine [ZDV]

Name of the Parameter	Method M_{1a}	Method M_{1c}	Method M_3	Method M_7	Method M_8
Maximum Wavelength λ_{max}	580	650	540	470	570
Beer's Law Limits µg/mL	5.0-30.0	5.0-30.0	5.0-30.0	5.0-30.0	6.667-40.0
Optimum Photometric Range µg/mL	15.0-25.0	10.0-20.0	12.0-20.0	10.0-20.0	13.33-26.66
Sandell's Sencitivity(µg/cm^2 / 0.001 Absorbance)	4.50E-02	5.95E-02	5.33E-02	8.47E-02	6.41E-02
Molar Absorptivity lt/mole/cm	5.89E+03	4.45E+03	4.54E+03	2.93E+03	4.27E+03
Slope (b)	2.21E-02	1.67E-02	1.70E-02	1.10E-02	1.60E-02
Intercept(a)	7.00E-03	7.33E-03	1.45E-02	6.27E-03	-2.87E-03
Standard Deviation on Slope(S_b)	3.08E-04	3.53E-04	5.20E-04	1.82E-04	1.32E-04
Standard Deviation on Intercept(S_a)	5.99E-03	6.88E-03	8.10E-03	3.54E-03	3.44E-03
Standard Error on Estimation(S_e)	8.39E-03	9.64E-03	1.13E-02	4.95E-03	4.81E-03
Correlation Coefficient (r)	0.9995	0.9987	0.9981	0.9994	0.9998
Limit of Detection (LOD) µg/mL	0.8148	1.2396	1.4302	0.9673	0.6445
Limit of Quantification (LOQ) µg/mL	2.7159	4.1320	4.7674	3.2244	2.1483

Table.4.05b

Optical and regression characteristics of the proposed methods for Zidovudine [ZDV]

Name of the Parameter	Method M_{10}	Method M_{11a}	Method M_{11b}
Maximum Wavelength λ_{max}	540	430	650
Beer's Law Limits µg/mL	4.0-24.0	3.333-20.0	3.33-20.00
Optimum Photometric Range µg/mL	8.0-20.0	10.0-16.667	10.0-20.0
Sandell's Sencitivity(µg/cm^2 / 0.001 Absorbance)	3.74E-02	0.0312	4.17E-02
Molar Absorptivity lt/mole/cm	6.81E+03	7.79E+03	6.05E+03
Slope (b)	2.55E-02	2.91E-02	2.26E-02
Intercept(a)	1.23E-02	8.33E-03	9.33E-03
Standard Deviation on Slope(S_b)	6.77E-04	6.15E-04	3.88E-04
Standard Deviation on Intercept(S_a)	1.05E-02	7.98E-03	5.03E-03
Standard Error on Estimation(S_e)	1.48E-02	1.12E-02	7.05E-03
Correlation Coefficient (r)	0.9980	0.9987	0.9994
Limit of Detection (LOD) µg/mL	1.2424	0.8217	0.6672
Limit of Quantification (LOQ) µg/mL	4.1414	2.7390	2.2241

Table.4.06a
Assay of Zidovudine [ZDV] in Pharmaceutical Formulations

Sample	Amount taken		Amount found in proposed methods			Reference Method	Recovery in proposed methods			
			M_{1a}	M_{1c}	M_3			M_{1a}	M_{1c}	M_3
VIRO-Z[a](tablets)	100mg	AVG	99.97	99.77	99.74	99.5	%REC	99.97	99.77	99.74
		SD	±0.640	±0.730	±0.598	± 0.69	%RSD	±0.640	±0.732	±0.740
		F	1.162	1.119	1.331					
		t	1.123	0.645	0.573					
ZIDO-H[b] (Capsules)	300mg	AVG	297.42	294.90	297.15	295.5	%REC	99.14	98.30	99.05
		SD	±3.12	± 2.78	±2.86	± 2.98	%RSD	±1.049	±0.942	±0.962
		F	1.096	1.149	1.085					
		t	1.063	0.332	0.913					

***Average of six determinations are considered, AVG=Average, SD=Standard deviation, F=F-test value, t=t-test value; Theoretical values at 0.05 level of confidence limit F=5.05, t=1.812.**

****%REC=% of Recovery, %RSD=%of Relative standard deviation; Recovery of 10.0mg added to the preanalyzed formulations (Average of six determinations)**

Table.4.06b
Assay of Zidovudine [ZDV] in Pharmaceutical Formulations

Sample	Amount Taken		Amount found in proposed methods			Reference Method	Recovery in proposed methods			
			M_7	M_8	M_{10}			M_7	M_8	M_{10}
VIRO-Z (tablets)	100mg	AVG	99.98	99.82	98.99	99.5	%REC	99.98	99.82	98.99
		SD	±066	±0.710	±0.750	± 0.69	%RSD	±0.660	±0.711	±0.758
		F	1.093	1.059	1.181					
		t	1.148	0.765	1.219					
ZIDO-H (Capsules)	300mg	AVG	296.40	297.03	296.97	295.5	%REC	98.8	99.01	98.99
		SD	±2.99	± 2.94	±2.96	± 2.98	%RSD	±1.008	±0.989	±0.996
		F	1.006	1.0136	1.013					
		t	0.498	0.847	0.813					

***Average of six determinations are considered, AVG=Average, SD=Standard deviation, F=F-test value, t=t-test value; Theoretical values at 0.05 level of confidence limit F=5.05, t=1.812.**

****%REC=% of Recovery, %RSD=%of Relative standard deviation; Recovery of 10.0mg added to the preanalyzed formulations (Average of six determinations)**

Table.4.06c
Assay of Zidovudine [ZDV] in Pharmaceutical Formulations

Sample	Amount taken		Amount found in proposed methods		Reference Method	Recovery in proposed methods		
			M_{11a}	M_{11b}			M_{11a}	M_{11b}
VIRO-Z (tablets)	100mg	AVG	98.77	98.97	99.5	%RECOV	98.77	98.97
		SD	±0.70	±0.632	± 0.69	%RSD	±0.709	±0.639
		F	1.029	1.191				
		t	1.745	1.267				
ZIDO-H (Capsules)	300mg	AVG	297.75	295.95	295.5	%RECOV	99.25	98.65
		SD	±3.05	± 2.89	± 2.98	%RSD	±1.024	±0.976
		F	1.047	1.063				
		t	1.245	0.249				

***Average of six determinations are considered, AVG=Average, SD=Standard deviation, F=F-test value, t=t-test value; Theoretical values at 0.05 level of confidence limit F=5.05, t=1.812.**

****%REC=% of Recovery, %RSD=%of Relative standard deviation; Recovery of 10.0mg added to the preanalyzed formulations (Average of six determinations)**

PART–B:VALIDATED RP-HPLC METHOD FOR ESTIMATION OF ZIDOVUDINE IN PHARMACEUTICAL FORMULATIONS

4.01-B: INTRODUCTION

Literature survey reveals that a very few HPLC methods [4-8] have been reported for the estimation of zidovudine in combined dosage forms. Reverse Phase High performance liquid chromatography (RP-HPLC) can be for the stability testing of drugs and for purposes of quality control of the finished product. It has the advantages of being sensitive, selective, rapid, accurate and reproducible. The present paper reports the development and validation of a new high performance thin layer chromatography (RP- HPLC) method for determination of zidovudine in **VIRO-Z(tablet).**

4.02-B: MATERIALS AND METHODS

i) Reagents and chemicals: Methanol of HPLC grade was procured from E.Merck (India) Ltd, Mumbai. Water HPLC grade was obtained from a Milli-QRO water purification system. A reference standard of Zidovudine was procured from Hetero labs, Hyderabad.

ii) Apparatus and chromatographic conditions: Chromatographic separation was performed on a Shimadzu® liquid chromatographic system equipped with a LC-10AT-vp solvent delivery system (pump), SPD M-10AVP - UV detector, Rheodyne 7725i injector with 50 mL loop volume. Class-VP 6.01 data station was applied for data collecting and processing (Shimadzu, Japan). Hyresil BDS C18 (250cm x 4.6 mm i.d., 5 μ) column with a mobile phase consisting of Methanol: water in the ratio 20:80 at a flow rate of 1.2mL.min^{-1} is used for the assay of zidovudine**.** Detection was

carried out at 265nm. The mobile phase was filtered through a 0.45µ membrane filter and degassed. The injection volume was 50mL and the analysis was performed at ambient temperature **Table.4.07,P.182.**

iii)Preparation of standard solution: The standard stock solution of zidovudine was prepared by dissolving 25mg of the drug in 25mL of the methanol to get 1.0mg/mL solution. Final working standard solution of 50µg/mL of zidovudine was prepared by diluting 5.0mL solution of the above solution to 10mL with mobile phase (methanol and water in the ratio 20:80 v/v). 5.0, 10.0, 15.0, 20.0 and 25.0µg/mL concentration of solutions were prepared and injected under operating chromatographic conditions.Calibration curves were constructed by plotting peak area versus concentration of zidovudine and the regression equation were calculated.

iv) Preparation of Marketed formulations: In case of marketed formulations, two accurately weighed tablets were crushed to a fine powder and an amount equivalent to 10mg of zidovudine was added into different 100mL volumetric flasks and volume was made up with methanol. The samples were filtered through a 0.45-µm membrane filter; different serial dilutions (5.0 – 25.0µg/mL) were made from this solution in 25mL volumetric flask and were injected for HPLC analysis.

Fig. 4.25. HPLC Chromatogram of zidovudine

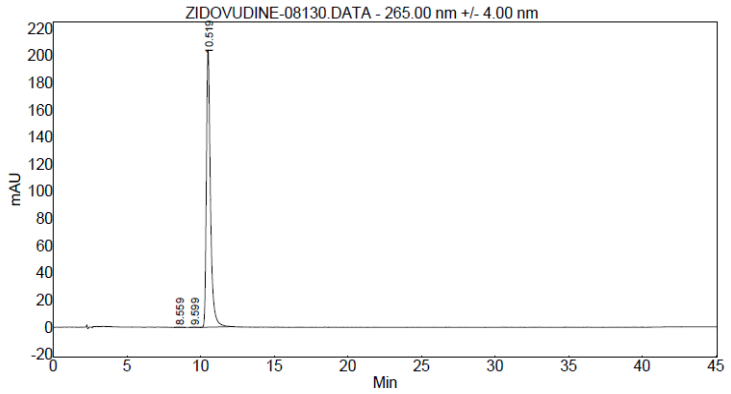


Table.4.07

Optimized chromatographic conditions

Chromatographic parameters	PEAK HPLC
Elution	Isocratic
Mobile phase	Metahnol:water in the ratio 20:80 v/v
Column	HYPERSIL BDS C-18 RP (4.6 mm i.d x 250 mm)
Flow rate	1.2 mL / min
Detection	UV at 265nm
Injection volume	20 µL
Temperature	Ambient
Retention time	10.519 minutes
Run time	15 minutes
Area	3964.57 mAU
Theoretical plates	7702.55
Pressure	17-20 Mpa

4.03-B: ASSAY METHOD

Under optimized chromatographic conditions as reported in **Table.4.07,P.187**, a steady baseline was recorded, when standard solution of zidovudine were injected and the chromatogram was recorded (**Fig.4.25,P.181**). The retention time of zidovudine was found to be 10.519min respectively. This procedure was repeated for the sample solutions obtained from the marketed formulations. The response factor (peak area ratio of standard peak area) of the standard solution and sample solution were calculated.

4.04-B: RESULTS AND DISCUSSION

i) Method development: The RP-HPLC procedure was optimized with a view to develop precise and stable assay method. Tthe pure drug zidovudine was runned in different mobile phase compositions with different C18 columns

(ODS,C18,5µ,250×4.6 mm) Phenomenex C18 column (25 cm x 4.6mm i.d., 5µ).The flow rate was also varied from 0.5 mL to 1.2mL.min. Finally, **HYPERSIL BDS C18 (ODS,C18,5µ,250×4.6 mm)** with a mobile phase of a mixture of methanol and water 20:80v/v at a flow rate of 1.2mL.min with a detection at 265nm gave sharp and symmetrical peak with retention time of 10.519 min for respectively. The typical chromatogram of sample solution of zidovudine is shown in **Fig.4.25,P.184**. The peak area ratio of standard and sample solutions was calculated. The assay procedures were repeated for six times and mean peak area and mean weight of standard drugs was calculated. The results of analysis shows that the amounts of drugs were in good agreement with the label claim of the formulation.

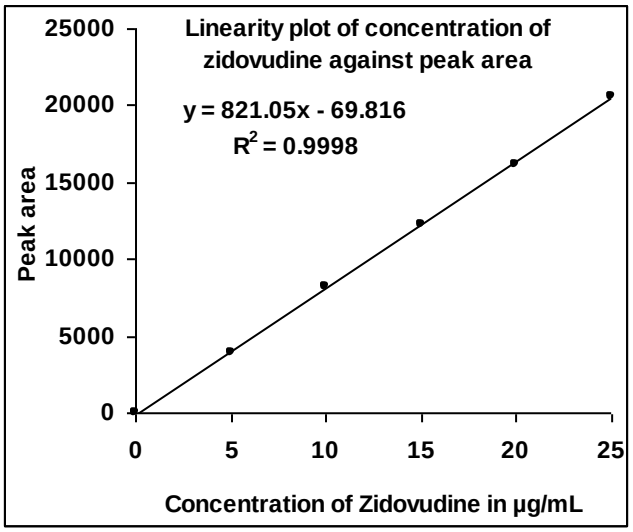
ii) Method Validation: The following parameter has been used to validate the developed RP- HPLC method for the estimation of zidovudine in pharmaceutical formulations.

a) Linearity and Range: The linearity of the method was determined at five concentration levels ranging from 5.0 to 25.0µg.mL^{-1} for zidovudine. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was **y = 0.0588x-0.0167 (R2=0.9913)** for zidovudine. The results show that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above (**Table. 4.08,P.184**). The calibration curve are shown in **Fig. 4.26,P.184**.

Table.4.08
Calibration of the RP HPLC for the estimation of Zidovudine

Concentration (µg.mL)	Area (mAU)
5	3964.57
10	8200.23
15	12200.12
20	16198.29
25	20596.45
Regression equation	Y = a X + b
Slope (a)	148.34
Intercept (b)	825.42
Correlation coefficient	0.9997

Fig.4.26. Linearity of zidovudine



b) Sensitivity [Limit of Detection and Limit of Quantification]: The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for zidovudine were found to be 5.0ng/mL respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10).

c) Precision: The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the interday variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage RSD were calculated and presented in **Table.4.09,P.185** From the data obtained, the developed RP-HPLC method was found to be precise.

Table.4.09
Precision data

Day (Conc.= 10 µg/mL)	**Precession Area Mean**	**R.S.D.**
Day- 1	77089.50	0.833
Day-2	89785.26	1.630

Averages of six determinations

d) Accuracy [Recovery studies]: Recovery study carried out for the drug was performed by spiking the known standard drug in powdered formulations. The assay procedure was repeated for standard and sample six times and mean peak area ratio and concentration of drug was calculated .The percentage of individual drug found in formulation, mean, standard deviation in formulation were calculated. The results of the recovery analysis were found to be 96.28 ± 1.120 to 100.08 ± 0.164 reported in **Table.4.10,P.186.** The results of analysis **(Table.4.11,P.186)** shows that the amounts of drug were in good agreement with the label claim of the formulation.

Table.4.10
Recovery studies of the proposed HPLC method

Labled amount µg/mL	Amount added µg/mL	Total amount µg/mL	Amount found µg/mL	% of Recovery	Mean
10	5	15	4.775	96.25%	
10	10	20	19.9132	98.91%	
10	15	25	25.088	100.8%	98.75%

All the values are the averages of three determinations

Table.4.11
Results of analysis (Recovery studies) of tablet containing zidovudine

Pharmaceutical formulation	Amount of zidovudine		% of recovery
	Labelled	Found	
VIRO-Z (tablet)	100 mg	102.328 mg	100.776 %

Average of three determinations

d) Ruggedness and Robustness: The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC2010AHT) and Water's Breeze HPLC by different operators using different

columns of similar type like ODS C18, Phenomenex Gemini C18 and Hichrom C18. Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed, are rugged and robust.

4.05-B: CONCLUSIONS

The RP-HPLC method for determining zidovudinein pharmaceutical formulations was developed using methanol and water (20:80, v/v) as mobile phase. The peak areas of the densitogram were quantified by densitometer at 265nm. The limit of detection and limit of quantitation were found to be $\mu g.mL^{-1}$, respectively. The calibration curve was linear over the range of 5.0 - 25.0$\mu g.mL^{-1}$. The correlation coefficient, slope and intercept were found to be **0.9993, 564.08(±30.08)** and **6838.63(±20.98)** respectively.The method was applied to the determination of zidovudine in **VIRO-Z[a](tablet)** with the average percentage recovery of 100.776 respectively. The proposed RP-HPLC method is simple, sensitive and accurate with good precision is suitable for routine analysis of this drug [zidovudine] in pharmaceutical formulations.

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