SECTION 2.0

DRUG PROFILE AND LITERATURE SURVEY

2.0.1. DRUG PROFILE

Zidovudine (ZDV), chemically known as 3\(^1\)-Azido-3\(^1\)-deoxy thymidine, was the first drug approved for the treatment of AIDS and HIV infection. It has the empirical formula C\(_{10}\)H\(_{13}\)N\(_5\)O\(_4\) and its molecular weight is 267.24.

ZDV was prepared by J.P. Horwitz et al.[1]. It has the following structure:

ZDV is a white to yellowish powder. It melts at about 124° C and exhibits polymorphism. It is soluble in water (25°C; 25 mg mL\(^{-1}\)) and soluble in alcohol. ZDV was the first drug approved for the treatment of AIDS and HIV infection. It is phosphorylated in the body to zidovudine triphosphate which is the active form which inhibits HIV replication. ZDV inhibits the key enzyme reverse transcriptase. ZDV is indicated for asymptomatic and symptomatic HIV disease [1a]. It is listed in United States Pharmacopoeia [2].
2.0.2. LITERATURE SURVEY OF TITRIMETRIC, SPECTROPHOTOMETRIC, HPLC, AND HPLC-MS-MS METHODS FOR ZIDOUDINE

Titrimetric methods

Literature survey revealed that no titrimetric method has ever been proposed for the determination of ZDV either in bulk drug or in dosage forms.

Visible spectrophotometric methods

No visible spectrophotometric method is available in literature.

HPLC methods

Only two methods are available in the literature. Dunge et al.[3] have reported a HPLC method for the determination of ZDV during stability studies. The assay was achieved on a C18 column utilizing water-methanol in the ratio of 77:23 as the mobile phase with a flow rate 1.4 mL min\(^{-1}\) and UV detection at 265 nm. The response is reported to be linear over the range 25-500 µg mL\(^{-1}\). Rebierre et al. [4] have described simultaneous determination of 19 antiretroviral molecules by two methods using C18 column and UV detection.

Other techniques

A few more other techniques such as UV-spectrophotometry [5], derivative differential UV spectrometry [6] and thermogravimetry, derivative thermogravimetry and differential thermal analyses [7] have been reported for the assay of ZDV in pharmaceuticals.

Methods for ZDV in urine

The literature survey reveals that the determinations of ZDV has mainly been focused on high performance liquid chromatography (HPLC) technique in body fluids such as human serum[8-11], human plasma[12-16], human plasma and urine[17], and rat plasma[18].

There are only few methods available for the determination of drug in body fluids using LC-MS. Kenny et al. [19] have reported simultaneous determination of zidovudine and lamivudine in human serum using HPLC with tandem mass spectrometry in the range of 2.5-2500 ng mL\(^{-1}\). One more method of simultaneous
determination of lamivudine and zidovudine concentrations in human seminal plasma using HPLC-tandem mass spectrometry was reported by Pereira et al. [20] using zidovudine as an internal standard. Moore et al. [21] reported the simultaneous quantification of the 5′-triphosphate metabolites in peripheral mononuclear blood cells of HIV infected patients by HPLC-MS-MS in the range of 50-45000 pg.

From the review of literature presented in the preceding paragraphs, it can be seen that no titrimetric and vis-spectrophotometric methods have ever been reported for ZDV. The reported HPLC method is less sensitive.

ZDV has two analytically useful functional groups, viz., hydroxyl and the amino groups. Targeting these functional groups, three titrimetric and seven spectrophotometric procedures have been developed by the author for its determination. The methods make use of bromate-bromide, N-bromosuccinimide and chloramine-T as oxidimetric reagents. Also, one HPLC method and one LC-MS-MS method have been developed for the assay of ZDV in pharmaceuticals and urine, respectively. The details of method development and validation of these methods are presented in this Chapter.
SECTION 2.1
TITRIMETRIC AND SPECTROPHOTOMETRIC METHODS FOR THE
DETERMINATION OF ZIDOVUDINE USING BROMATE-BROMIDE
MIXTURE AND TWO DYES, METHYL ORANGE AND INDIGO CARMINE*

2.1.1.0 INTRODUCTION

In acid solution bromate is a strong oxidant (E° = 1.52V); bromide is formed in
the first stage of the reaction and then reacts with excess bromate to yield free
bromine.

\[
\begin{align*}
\text{BrO}_3^- + 6 \text{H}^+ + 6e^- & \rightarrow \text{Br}^- + 3\text{H}_2\text{O} \\
\text{BrO}_3^- + 5 \text{Br}^- + 6 \text{H}^+ & \rightarrow 3\text{Br}_2 + 3\text{H}_2\text{O}
\end{align*}
\]

It is usual to add bromide to the test solution before the titration or to include
in the standard bromate solution so that only the second reaction is involved.

An acidified mixture of bromate and bromide actually behaves as an
equivalent solution of bromine. Thus the stable bromate-bromide solution serves for
the extemporaneous preparation of a standard solution of bromine. Aqueous bromine
solutions are unstable because of high vapour pressure of bromine.

The oxidizing action of bromate appears to have been noted by Balard [22],
but the first application of bromate as titrimetric reagent was due to Koppeschaar [23],
who used it in his well-known bromination procedure for the determination of phenol.
Kratschmer [24] first recommended the use of bromate as an oxidimetric reagent.
Since then the reagent in combination with bromide has found wide application in
chemical analysis [25-27] including substances of pharmaceutical importance.

Bromate–bromide mixture in acid medium has been used in the past for the
direct titrimetric assay of wide-ranging pharmaceuticals [25-27], with visual,
electrometric or photometric detection of end point. Some of the examples reported in
the last three decades include amethoclain hydrochloride [28], phenolic steroids [29],
isonicotinic acid hyrazide [30], ledol [31], ascorbic acid [32-34], aminosalicylic acid
[35], citral [36], thiamine hydrochloride [37,38], oxyphenbutazone [39], cimetidine
[40], secobarbital [41], carbimazole [42], albendazole [43], salbutamol sulphate [44],
captopril [45], ranitidine [46], adrenergic drugs [47], sulphonamide [48],
tuberulostatic drugs [49], and nizatidine [50].

*This work has been published in *Bulg. Chem. Commun.*, 2007, 37(1), 53-59
Potassium bromate has also been a useful reagent for the direct spectrophotometric determination of pharmaceuticals like adrenergic drugs [47], sulphonamide [48], histidine [51], phenothiazines [52-56], benzylpenicillin [57], vitamin C [58], acetylinic hypnotics [59], diclofenac sodium [60], and isoniazid, trimiprazine tartrate, pyridoxine, phenothiazines and captopril [61].

Many dyes are irreversibly oxidised/destroyed to colourless products by oxidising agents in acid medium [26] and this observation has been exploited for the indirect spectrophotometric determination of many oxidisable pharmaceuticals [62-66]. Methyl orange and indigo carmine are irreversibly bleached by insitu generated bromine [26], and the bleaching action has successfully been utilised for the indirect spectrophotometric assay of a wide ranging pharmaceuticals such as albendazole [43], salbutamol sulphate [44], captopril [45], ranitidine [46], famotidine [67], chlorpromazine [68], astemizole [69], prochlorperazine [70] mebrophenhydramine [71], felodipine [72], amoxycillin [73] trifluoperazine [74], frusemide [75], cyproheptadine [76], metaprolol tartrate [77] and amlodipine besylate [78].

From the literature survey presented in Section 2.0.2 and from the foregoing paragraphs, it is clear that bromate-bromide reagent has not been used for the assay of ZDV in pharmaceuticals. In this section, simple and sensitive titrimetric and spectrophotometric methods are described for the determination of ZDV using bromate-bromide mixture and two dyes, methyl orange and indigo carmine as reagents. In titrimetry, ZDV is treated with a measured excess of bromate-bromide in acid medium followed by iodometric back titration of the residual bromine. In spectrophotometric methods, the unreacted bromine is determined by reacting with a fixed amount of either methyl orange or measuring the absorbance at 520 nm (method A) or indigocarmine and measuring the absorbance at 610 nm (method B).
2.1.2. EXPERIMENTAL

2.1.2.1. Apparatus

A Systronics model 106 digital spectrophotometer with 1-cm matched quartz cells was used for all absorbance measurements.

2.1.2.2. Reagents and materials

All the reagents used were of analytical-reagent grade and distilled water was used throughout investigation.

**Bromate-bromide mixture.** A standard solution equivalent to 2mM KBrO$_3$ - 20mM KBr was prepared by dissolving accurately weighed 3.34 g of KBrO$_3$ (Sarabhai M. chemicals, Baroda, India) and 15.0 g of KBr (S.d. Fine Chem. Ltd., Mumbai, India) in water and diluting to 1 litre in a volumetric flask, and used in titrimetric assay.

**Bromate-bromide mixture (10 and 30 µg mL$^{-1}$ in KBrO$_3$).** A stock standard solution equivalent to 1000 µg mL$^{-1}$ KBrO$_3$ and a large excess of KBr was first prepared by dissolving accurately weighed 100 mg of KBrO$_3$ and 1 g of KBr in water and diluting to the mark with water in a 100 mL calibrated flask. This was diluted stepwise to obtain working concentrations containing 10 and 30 µg mL$^{-1}$ KBrO$_3$ for use in spectrophotometric method A and method B, respectively.

**Hydrochloric acid (5M).** Concentrated hydrochloric acid (S.D. Fine Chem., Mumbai, India; sp. gr. 1.18) was diluted appropriately with water to get 5M for spectrophotometric work and subsequently diluted to 1M for titrimetric work.

**Methyl orange (50 µg mL$^{-1}$).** A 500 µg mL$^{-1}$ dye solution was first prepared by dissolving accurately weighed 58.8 mg of dye (S.d. Fine Chem., Mumbai, India, 85% dye content) in water and diluting to 100 mL in a calibrated flask and filtered using glass wool. It was then diluted to obtain a working concentration of 50 µg mL$^{-1}$.

**Indigo carmine (200 µg mL$^{-1}$).** A 1000 µg mL$^{-1}$ stock standard solution was first prepared by dissolving accurately weighed 112 mg of dye (S.d. Fine Chem., Mumbai, India, 90% dye content) in water and diluting to volume in a 100 mL calibrated flask. The solution was then diluted 5-fold to get the working concentration of 200 µg mL$^{-1}$.

**Sodium thiosulphate (0.012 M).** Prepared by dissolving ~ 3 g of the chemical (SISCO chem. Industries, Mumbai) in 1 litre of water, and standardized iodometrically using pure potassium dichromate [79].
**Starch indicator (1%).** One g of soluble starch (S. d. Fine Chem. Ltd., Mumbai) was made into a paste with little water, and poured into 100 mL boiling water; boiled for 1 min and cooled.

**Potassium iodide (10%).** Prepared by dissolving 10 g of the chemical (S. d. Fine Chem. Ltd., Mumbai) in 100 mL of water.

**Standard solution of zidovudine.** Pharmaceutical grade zidovudine certified to be 99.8 % pure was received from Cipla Ltd, Mumbai, India, as gift, and was used as received. A stock standard solution equivalent to 1 mg mL\(^{-1}\) ZDV was prepared by dissolving accurately weighed 250 mg of pure drug in water and made up to the mark with water in a 250 mL calibrated flask, and was used for titrimetric work. The stock solution (1000 µg mL\(^{-1}\) ZDV) was diluted appropriately with water to get working concentrations of 10 and 40 µg mL\(^{-1}\) for use in spectrophotometric method A and method B, respectively. The standard solutions were kept in amber coloured bottle and stored in a refrigerator when not in use.

### 2.1.3. PROCEDURES

#### 2.1.3.1. Titrimetry

A 10.0 mL aliquot of standard drug solution containing 2-10 mg of ZDV was measured accurately and transferred into a 100 mL Erlenmeyer flak. The solution was acidified by adding 5 mL of 1 M hydrochloric acid after which 10 mL of bromate-bromide mixture (2mM in KBrO\(_3\)) was added by means of a pipette and the flask was stoppered. The content was mixed well and let stand for 5 min with periodic swirling. Finally, the sides of the flask and the stopper were washed with water into the flak; 5 mL of 10% potassium iodide solution were added and the liberated iodine titrated with 0.012 M sodium thiosulphate to a starch end point. A blank titration was performed omitting the drug solution. The amount of the drug present in the measured aliquot was calculated from the amount of KBrO\(_3\) that has reacted with the drug.

#### 2.1.3.2. Spectrophotometry using methyl orange (method A)

Different aliquots (0.5 ---- 5.0 mL) of a standard 10 µg mL\(^{-1}\) ZDV solution were transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was adjusted to 5 mL by adding adequate quantity of water. To each flask were added 2 mL of 5M HCl and 1 mL of bromate-bromide solution (10 µg mL\(^{-1}\) in KBrO\(_3\)), the last being measured accurately. The flasks were stoppered,
content mixed and let stand for 5 min with occasional shaking. Finally, 1 mL of 50 µg mL\(^{-1}\) methyl orange solution was added (accurately measured) and the volume was diluted to the mark with water and mixed well. The absorbance of each solution was measured at 520 nm against a reagent blank after 10 min.

2.1.3.3. Spectrophotometry using indigo carmine (method B)

Varying aliquots (0.5----5.0 mL) of a standard 40 µg mL\(^{-1}\) ZDV solution were transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was brought to 5 mL by adding water. To each flask were added 1 mL of 5 M hydrochloric acid and 1.5 mL of bromate-bromide solution (30 µg mL\(^{-1}\) in KBrO\(_3\)) by means of a micro burette. The content was mixed well and the flasks were kept aside for 10 min with intermittent shaking. Finally, 1 mL of 200 µg mL\(^{-1}\) indigo carmine solution was added to each flask, the volume was diluted to the mark with water, mixed well and absorbance measured against a reagent blank at 610 nm after 5 min.

In either spectrophotometric method, a standard graph was prepared by plotting the absorbance versus the concentration of ZDV. The concentration of the unknown was read from the calibration graph or computed from the regression equation derived using Beer’s law data.

**Procedure for formulations**

A quantity of the finely ground tablet/capsule powder equivalent to 100 mg of ZDV was accurately weighed into a 100 mL calibrated flask, 60 mL of water added and shaken for 20 min; the volume was finally diluted to the mark with water, mixed well and filtered using a Whatman No. 42 filter paper. The first 10 mL portion of the filtrate was discarded, and a convenient aliquot (say 5 mL) was taken for assay by titrimetric procedure. The filtrate (1000 µg mL\(^{-1}\) ZDV) was appropriately diluted with water to get 10 and 40 µg mL\(^{-1}\) ZDV concentrations and analysed by spectrophotometric methods by taking convenient aliquots (1 - 3 mL). The results are presented in Table 2.1.3.
2.1.4. RESULTS AND DISCUSSION

2.1.4.1. Method development

Titrmetry: The quantitative nature of reaction between ZDV and in situ generated bromine was checked by treating 2 - 10 mg of ZDV with a measured excess of bromate-bromide mixture in acid medium and determining the surplus bromine iodometrically. In the range studied (2 - 10 mg), the reaction stoichiometry was found to be 1:0.333(ZDV: KBrO₃). The possible reaction scheme is shown in Fig. 2.1.1.

\[
\text{BrO}_3^- + 5 \text{Br}^- + 6 \text{H}^+ \rightarrow 3 \text{Br}_2 + 3 \text{H}_2\text{O}
\]

\[
\text{Zidovudine} + \text{Known excess of in situ Bromine} \xrightarrow{\text{H}^+} \text{Reaction product + Unreacted bromine, determined iodometrically}
\]

**Fig. 2.1.1.** Possible Reaction scheme

The reaction stoichiometry was found to be unaffected in the presence of 3 to 5 mL of 1M HCl in a total volume of 23 to 25 mL, and 5 mL was chosen as the optimum volume. The bromination reaction was found to be complete in 5 min and contact time upto 15 min had no effect on the stoichiometry or the results. A 10 mL volume of 2 mM bromate solution in the presence of a large amount of bromide was found adequate for quantitative bromination of ZDV in the range investigated. The linearity between the amount of drug and titration end point is apparent from the calculated correlation coefficient of –0.9953 suggesting that the reaction between ZDV and bromine proceeds stochiometrically in the ratio 1:0.333.
Spectrophotometry: The proposed spectrophotometric methods are indirect and are based on the determination of the residual bromine (*insitu* generated) after allowing the reaction between ZDV and a measured amount of bromine to be complete. The surplus bromine was determined by reacting it with a fixed amount of either methyl orange or indigo carmine dye. The methods make use of bleaching action of bromine on the dyes, the decolouration being caused by the oxidative destruction of the dyes.

\[
\text{ZDV + Known excess of Br}_2 \xrightarrow{H^+} \text{Reaction Product + Unreacted bromine}
\]

Unreacted bromine + Methyl orange \rightarrow Absorbance measured at 520 nm (method A)

Unreacted bromine + Indigo Carmine \rightarrow Absorbance measured at 610 nm (method B)

ZDV when added in increasing concentrations to a fixed concentrations of *insitu* generated bromine consumes the latter proportionately and there occurs a concomitant fall in the concentration of bromine. When a fixed concentration of dye is added to decreasing concentrations of bromine, a concomitant increase in the concentration of dye results. Consequently, a proportional increase in the absorbance at the respective $\lambda_{\text{max}}$ is observed with increasing concentration of ZDV (Fig. 2.1.2 and 2.1.3).

![Beer's law curve for method A](image)

**Fig. 2.1.2.** Beer’s law curve for method A
Preliminary experiments were performed to fix the upper concentrations of the dyes that could be determined spectrophotometrically, and these were found to be 5 and 20 µg mL⁻¹ for methyl orange and indigo carmine respectively. A bromate concentration of 1µg mL⁻¹ in the presence of excess of bromide was found to bleach the red colour due to 5 µg mL⁻¹ methyl orange whereas 4.5 µg mL⁻¹ bromate was required to destroy the blue colour due to 20 µg mL⁻¹ indigo carmine. Hence, different amounts of ZDV were reacted with 1 mL of 10 µg mL⁻¹ KBrO₃ in method A and 1.5 mL of 30 µg mL⁻¹ KBrO₃ in method B followed by determination of residual bromine as described under the respective procedure.

For both the steps, i.e., the reaction between ZDV and bromine, and the determination of the latter by reacting with the dye, HCl medium was found to be ideally suited. The absorbance of the dye was not affected in 0.1 to 0.3 M HCl concentration. However, 2 mL of 5M HCl and 1mL of 5M HCl were selected for bromination of drug in method A and method B, respectively. The bromination reaction was found to be complete in 5 min and contact times upto 30 min had no effect on the absorbance of dyes. The absorbance of either dye solution even in the presence of brominated drug product was found to be stable for several days.

2.1.4.2. Method Validation

Linearity Range and Sensitivity

Linearity was observed over the concentration ranges 0.5 -5.0 and 2.0 -20 µg mL⁻¹ ZDV for method A and method B, respectively, confirming the adherence of the
system to Beer’s law. Absorbance and concentration data were subjected to linear least-squares regression analysis to calculate the calibration equation and correlation coefficients (Table. 2.1.1). The graphs showed negligible intercept as described by the regression equation:  
\[ Y = a + bX \]
(\text{where } Y = \text{absorbance of 1-cm layer of solution}; a = \text{intercept}; b = \text{slope and } X = \text{concentration in } \mu g\text{ mL}^{-1}). Regression analysis of the Beer’s law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient(r) for each system and the values are presented in Table 2.1.1.

The limits of detection (LOD) and quantitation(LOQ) are calculated according to ICH guidelines [80] using the equation:
\[
\text{LOD} = 3\sigma/S \quad \text{and} \quad \text{LOQ} = 10\sigma/S
\]
where \(\sigma\) is the standard deviation of replicate determination values under the same conditions for the sample analysis in the absence of the analyte and \(S\) is the slope of the calibration graph. The low values of LOD and LOQ indicate that the sensitivity of the methods is adequate. The molar absorptivity and Sandell sensitivity values are also given in Table 2.1.1.

**Table 2.1.1.** Analytical and regression parameters of spectrophotometric methods.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method A</th>
<th>Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\lambda_{\text{max}}, \text{ nm})</td>
<td>520</td>
<td>610</td>
</tr>
<tr>
<td>Beer’s law limits, (\mu g\text{ mL}^{-1})</td>
<td>0.5 – 5.0</td>
<td>2.0 – 20.0</td>
</tr>
<tr>
<td>Molar absorptivity, (\text{L mol}^{-1}\text{ cm}^{-1})</td>
<td>(4.0\times10^4)</td>
<td>(8.6\times10^3)</td>
</tr>
<tr>
<td>Sandell sensitivity, (\mu g\text{ cm}^{-2})</td>
<td>0.0067</td>
<td>0.031</td>
</tr>
<tr>
<td>Limit of detection, (\mu g\text{ mL}^{-1})</td>
<td>0.07</td>
<td>0.27</td>
</tr>
<tr>
<td>Limit of quantification, (\mu g\text{ mL}^{-1})</td>
<td>0.20</td>
<td>0.82</td>
</tr>
<tr>
<td>Regression equation, (Y^*)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.0110</td>
<td>-0.0220</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.1450</td>
<td>0.0350</td>
</tr>
<tr>
<td>Correlation coefficient, (r)</td>
<td>0.9992</td>
<td>0.9997</td>
</tr>
<tr>
<td>(S_a)</td>
<td>0.065</td>
<td>0.006</td>
</tr>
<tr>
<td>(S_b)</td>
<td>0.011</td>
<td>0.001</td>
</tr>
</tbody>
</table>

\(^*Y = a+bX,\) where \(Y\) is the absorbance and \(X\) concentration in \(\mu g\text{ mL}^{-1}\).

\(S_a=\) Standard deviation of intercept ; \(S_b=\) Standard deviation of slope.
Intra-day and inter-day accuracy and precision

The precision of the methods was evaluated in terms of intermediate precision (intra-day and inter-day). Three different concentrations of ZDV (within the Beer’s law range) were analysed in seven replicates during the same day (intra-day precision) and five consecutive days (inter-day precision) by preparing all solutions afresh each day. The RSD values of intra-day and inter-day precision studies for both methods showed that the precision was satisfactory (Table 2.1.2).

The accuracy was evaluated as percentage relative error between the measured mean concentrations and added concentrations of ZDV (Bias, %). The results obtained for three different concentrations are shown in Table 2.1.2, from which it is clear that the accuracy is fairly good in both the methods.

<table>
<thead>
<tr>
<th>Method*</th>
<th>ZDV taken</th>
<th>Intra-day accuracy and Precision a</th>
<th>Inter-day accuracy and precision b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ZDV taken</td>
<td>Intra-day accuracy and Precision a</td>
<td>Inter-day accuracy and precision b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZDV found RE, % RSD, %</td>
<td>ZDV found RE, % RSD, %</td>
</tr>
<tr>
<td>Titrimetry</td>
<td>3.0</td>
<td>2.96 1.33 1.69</td>
<td>2.97 1.00 1.72</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>4.91 1.80 1.22</td>
<td>4.93 1.40 1.22</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>7.84 2.00 1.79</td>
<td>7.85 1.88 1.78</td>
</tr>
<tr>
<td>Spectrophotometric method A</td>
<td>1.5</td>
<td>1.49 0.67 0.67</td>
<td>1.47 2.00 0.68</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>3.51 0.29 0.57</td>
<td>3.48 0.57 0.57</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>4.59 2.00 1.09</td>
<td>4.43 1.56 1.13</td>
</tr>
<tr>
<td>Spectrophotometric method B</td>
<td>6.0</td>
<td>5.85 2.50 0.68</td>
<td>5.86 2.33 0.69</td>
</tr>
<tr>
<td></td>
<td>14.0</td>
<td>13.92 0.57 2.01</td>
<td>13.93 0.50 2.01</td>
</tr>
<tr>
<td></td>
<td>18.0</td>
<td>17.57 2.39 1.59</td>
<td>17.60 2.22 1.59</td>
</tr>
</tbody>
</table>

RE, relative error; RSD, relative standard deviation.
*In titrimetry, ZDV taken/found are in mg, while in the spectrophotometric methods, the quantities are in µg mL⁻¹.
a. n=7; b. n=5.

Robustness and ruggedness

To evaluate the method robustness, two experimental variables such as acid concentration and reaction time were slightly altered, and the same were found to have no significant effect on the precision of the methods when studies were made on a single concentration of ZDV. The percent RSD values were ≤ 4 in both instances.
The ruggedness of the methods was assessed by calculating the RSD for results obtained by performing the analysis using three different instruments and by three different persons. The inter-instrumental RSD values ranged from 2.5-4.5% whereas the inter-personal RSD values varied from 2.5-3.5% for three concentrations of ZDV employed for accuracy and precision. The study suggested that the methods were robust as well as rugged.

**Selectivity**

To evaluate the usefulness of the methods, the effect of excipient and additives, which often accompany ZDV in tablet/capsule, was studied. A synthetic mixture with the composition: ZDV (100 mg); NaCl (200 mg); talc (50 mg); starch (150 mg); lactose (200 mg); titanium dioxide (50 mg); sodium alginate (50 mg) and magnesium stearate (25 mg), was prepared and the active component (ZDV) was extracted with water as described under procedure for tablets and analysed as recommended procedure. The percentage recoveries (n=5) of ZDV was found to be 98.3±3.2 for titrimetry, 98.5±1.5 for spectrophotometric method A and 98.1±2.2 for spectrophotometric method B, indicating high selectivity for determining the studied drug in formulations.

**Application to tablet/capsule analysis**

In India, six brands of tablets/capsules in 100 mg and 300 mg doses are commercially available. The validity of the methods was checked by applying them to assay in two brands of capsules and tablets. Table 2.1.3 gives the results of assay and reveals that there is close agreement between the results obtained by the proposed methods and the label claim. The results were also compared statistically with those obtained by a reference method [5] (The assay was performed at concentration level of 10 µg mL⁻¹ in water and scanned from 190-700 nm and absorbance measured at 268 nm), by applying Student’s t-test for accuracy and F-test for precision. At the 95% confidence level, the calculated t- and F-values did not exceed the tabulated values (t = 2.77 and F = 6.39) suggesting that the proposed methods are as accurate and precise as the reference method.
Table 2.1.3. Results of determination of zidovudine in formulations and statistical comparison with the reference method.

<table>
<thead>
<tr>
<th>Capsule/tablet Brand name&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Nominal amount, mg</th>
<th>% found* ± SD</th>
<th>Reference method</th>
<th>Titrimetry</th>
<th>Spectrophotometric method A</th>
<th>Spectrophotometric method B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>99.91±0.50</td>
<td>100.5±1.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>t=2.70</td>
<td>t=0.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F=1.12</td>
<td>F=4.47</td>
</tr>
<tr>
<td>VIRO-Z&lt;sup&gt;a&lt;/sup&gt; (Tablets)</td>
<td>100</td>
<td>100.8±0.53</td>
<td></td>
<td>99.95±0.66</td>
<td>100.2±1.46</td>
<td>99.95±0.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>t=1.20</td>
<td>t=0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F=1.02</td>
<td>F=3.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100.2±1.02</td>
<td>100.1±1.25</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>t=1.20</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F=2.00</td>
<td>F=3.17</td>
</tr>
<tr>
<td>ZIDO-H&lt;sup&gt;b&lt;/sup&gt; (Capsules)</td>
<td>300</td>
<td>100.6±0.56</td>
<td></td>
<td>100.2±0.91</td>
<td>100.8±1.43</td>
<td>99.56±1.33</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>F=2.64</td>
<td>F=6.52</td>
</tr>
</tbody>
</table>

*Mean value of five determinations.

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Tabulated t-value at 95% confidence level is 2.77
Tabulated F-value at 95% confidence level is 6.39.

Recovery Study

The accuracy and validity of the proposed methods were further ascertained by performing recovery experiments. Pre-analysed tablet/capsule powder was spiked with pure ZDV at three different levels and the total was found by the proposed methods. Each determination was repeated three times. The recovery (Table 2.1.4.) of pure drug added was quantitative and revealed that co-formulated substances did not interfere in the determination.
Table 2.1.4. Results of recovery experiments by standard addition method.

<table>
<thead>
<tr>
<th>Formulation studied</th>
<th>Titrimetry</th>
<th>Spectrophotometric method A</th>
<th>Spectrophotometric method B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ZDV in tablet, mg</td>
<td>Pure ZDV added, mg</td>
<td>Total found, mg</td>
</tr>
<tr>
<td>VIIRO-Z 100</td>
<td>2.0</td>
<td>2.0</td>
<td>4.04</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>4.0</td>
<td>5.99</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>8.0</td>
<td>10.12</td>
</tr>
<tr>
<td>ZIDO-H 300</td>
<td>2.0</td>
<td>2.0</td>
<td>3.96</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>4.0</td>
<td>5.95</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>8.0</td>
<td>10.07</td>
</tr>
</tbody>
</table>

*Mean value of three determinations.
SECTION 2.2
TITRIMETRIC AND SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF ZIDOVUDINE USING N-BROMOSUCCINIMIDE BASED ON REDOX REACTION*

2.2.1. INTRODUCTION

N-bromosuccinimide (NBS) whose structure is given below is perhaps the most important positive bromine containing organic compound.

\[
\begin{array}{c}
\text{O} \\
\text{N} \\
\text{O} \\
\text{Br}
\end{array}
\]

The compound was developed basically for use as a synthetic reagent but because of the high yields of the products obtainable in its reactions, it has been adapted for use in the determination of many organic compounds. NBS was first synthesized by Seliwanow [81] in 1893. Ziegler and his coworkers [82] made detailed studies of its applications for allylic bromination. NBS, under different conditions, reacts with alkenes to add bromine to the double bond or to act as a source of hypobromous acid in aqueous solution. It was, therefore, used extensively as a brominating and oxidizing agent.

The work on the analytical applications of NBS has been more of recent. In the monograph compiled by Mathur and Narang [83], a survey of the analytical applications of NBS for the determination of organic compounds is given. The analytical applications of NBS are broadly based on bromination and oxidation reactions. These reactions have found extensive applications in the determination of a variety of organic compounds including those of pharmaceutical interest. The utility of NBS in pharmaceutical analysis can be gauged by the fact that nearly 40 different substances have been determined between 1991 and 2008 by employing this reagent in titrimetric and spectrophotometric analysis.

*This work has been published in Proc. Natl. Acad. Sci. India, 2007, 77(A) IV, 301-308
NBS has been a valuable oxidimetric reagent for the determination of many organic and inorganic substances, and has been widely used in assay of several pharmaceutical substances.

Phenothiazines [84-87], ranitidine [88], azapropazone [89] antiinflammatory drugs[90], ethorvynol [91], clotrimazole [92], pyridoxine hydrochloride [93], pyroxicam and diclofenac sodium [94] benzodiazepines [95], thioxanthene derivatives [96], antazoline HCl and phenylepherine HCl [97], acetylenic hypnotics [98] isomiazid [99,100], salbutamol sulphate [101], cemitidine [102] and oxyphenbutazone [103] are some of the pharmaceutical substances which have been assayed titrimetrically in recent years with visual, electrometric and thermometric end point detection using NBS as the oxidimetric reagent. Likewise, NBS either alone or in combination with some other reagents has been widely applied in the direct or indirect spectrophotometric determination of several pharmaceutical substances which include phenothiazines [84-86], chindamycin [104], sulphonamides [105] diclofenac sodium [106], propanolol [107,108], piperezine [109], benzo diazepines [110], sulphonamide diuretis [111], paparerine HCl [112], nifartimox [113], tetracycline, propanolol, isoniazid hydrazide and nifartimox [114], diltiazem HCl [115], cyproheptadine HCl [116] ascorbic acid [117], astemizole [118], amoxycilin and cafadroxil [119], and oxprenolol [120], prozorin HCl [121] omeprazole [122,123], azapropazone [124], cefotaxime sodium [125], gatifloxacin [126], ranitidine [127], meloxicam [128] lamivudine [129], albendazole [130], salbutamol sulphate [131], metaprolal tartrate [132], nitrofurazone [133] and cimetidine, famotidine, nizatidine, ranitidine [134].

Further, from the review of the application of N-bromosucinimide as an oxidimetric reagent in pharmaceutical analysis and from the methods reported for ZDV reviewed in section 2.0.2, it is seen that NBS has not been employed for the analysis of ZDV. In this section, simple and sensitive titrimetric and spectrophotometric methods are described for the determination of ZDV using NBS and two dyes, methyl orange and indigo carmine as reagents. In titrimetry, ZDV is treated with a measured excess of NBS in acid medium followed by iodometric back titration. In spectrophotometric methods, the unreacted oxidant is determined by reacting with a fixed amount of either methyl orange or measuring the absorbance at 520 nm (method A) or indigocarmine and measuring the absorbance at 610 nm (method B). These findings are presented in this Section 2.2.
2.2.2.0. EXPERIMENTAL

2.2.2.1. Apparatus

The instrument used in the same as the one described in section 2.1.2.1.

2.2.2.2. Reagents and materials

All the reagents used were of analytical-reagent grade and distilled water was used throughout.

**N-bromosuccinimide (NBS):** An approximately 0.01 M solution was prepared by dissolving about 1.8 g of NBS (SRL Research Chemicals, India) in water with the aid of heat and diluted to one litre with water. The solution was standardized iodometrically [135] and kept in an amber coloured bottle stored in a refrigerator and used for titrimetry. It was diluted appropriately to get 80 and 340 µg mL⁻¹ NBS for use in spectrophotometric method A and method B respectively.

**Hydrochloric acid:** Concentrated hydrochloric acid (S.D. Fine Chem., Mumbai, India; sp. gr. 1.18) was diluted appropriately with water to get 2 M acid for titrimetric work and subsequently diluted to 1M for spectrophotometric work.

**Sodium thiosulphate:** A 0.01 M thiosulphate solution was prepared by dissolving 5 g of the chemical (S. d. Fine Chem. Ltd., Mumbai, India) in one litre of water for use in titrimetric method.

The preparation of methyl orange (50 µg mL⁻¹), indigocarmine (200 µg mL⁻¹), potassium iodide (10%), and starch indicators is described in section 2.1.2.2.

**Preparation of standard ZDV solution**

A stock standard solution containing 1 mg mL⁻¹ ZDV was prepared as described in Section 2.1.2.2 and used in titrimetric analysis. This was appropriately diluted to yield working concentrations of 20 and 50 µg mL⁻¹ for spectrophotometric method A and method B, respectively.

2.2.3.0. PROCEDURES

2.2.3.1. Titrimetry

A 10.0 mL aliquot of standard drug solution containing 4-15 mg of ZDV was measured accurately and transferred into a 100 mL Erlenmeyer flak. The solution was acidified by adding 2 mL of 2 M hydrochloric acid after which 10 mL of standard
NBS (0.01M) was added by means of a pipette. The content was mixed well and let stand for 10 min with periodic swirling. Finally, 5 mL of 10% potassium iodide solution was added and the liberated iodine titrated with 0.01 M sodium thiosulphate to a starch end point. A blank titration was performed omitting the drug solution. The amount of the drug present in the measured aliquot was calculated from the volume of NBS reacted with the drug.

2.2.3.2. Spectrophotometry using methyl orange (method A)

Different aliquots (0.25 ---- 3.0 mL) of a standard 20 µg mL$^{-1}$ ZDV solution were transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was adjusted to 3 mL by adding adequate quantity of water. To each flask were added 1 mL each of 1M HCl and 1 mL of NBS solution (80 µg mL$^{-1}$), the last being measured accurately. The flasks were stoppered, content mixed and let stand for 5 min with occasional shaking. Finally, 1 mL of 50 µg mL$^{-1}$ methyl orange solution was added (accurately measured) and the volume was diluted to the mark with water and mixed well. The absorbance of each solution was measured at 520 nm against a reagent blank after 10 min.

2.2.3.3. Spectrophotometry using indigo carmine (method B)

Varying aliquots (0.5----4.0 mL) of a standard 50 µg mL$^{-1}$ ZDV solution were transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was brought to 5 mL by adding water. To each flask were added 1 mL of 1 M hydrochloric acid and 1.0 mL of NBS solution (340 µg mL$^{-1}$ ) by means of a micro burette. The content was mixed well and the flasks were kept aside for 5 min with intermittent shaking. Finally, 1 mL of 200 µg mL$^{-1}$ indigo carmine solution was added to each flask, the volume was diluted to the mark with water, mixed well and absorbance measured against a reagent blank at 610 nm after 10 min.

In either spectrophotometric method, a standard graph was prepared by plotting the absorbance versus the concentration of ZDV. The concentration of the unknown was read from the calibration graph or computed from the regression equation derived using Beers’ law data.
Procedure for dosage forms

Tablet/capsule extract equivalent to 1 mg mL\(^{-1}\) ZDV was prepared as described in section 2.1.3 and a convenient aliquot (say 5 mL) of the extract was analyzed by titrimetry as described earlier. The filtrate (1000 µg mL\(^{-1}\) in ZDV) was diluted with water to obtain 20 and 50 µg mL\(^{-1}\) concentrations and subjected to analysis by spectrophotometric methods following the procedures described already.

**2.2.4.0. RESULTS AND DISCUSSION**

**2.2.4.1. Method development**

In titrimetry, the quantitative nature of reaction between ZDV and NBS was checked by treating 4 to 15 mg of ZDV with a measured excess of NBS in acid medium and determining the surplus oxidant iodometrically. In the range studied (4 to 15 mg), the reaction stoichiometry was found to be 1:2 (ZDV: NBS).

\[
\text{ZDV} + \text{Known excess of NBS} + H^+ \rightarrow \text{Reaction product} + \text{Unreacted NBS determined iodometrically}
\]

The reaction stoichiometry was found to be unaffected in the presence of 1 to 3 mL of 2M HCl in a total volume of 25 mL, and 2 mL was chosen as the optimum volume. The oxidation reaction was found to be complete in 5 min and contact time up to 20 min had no effect on the stoichiometry or the results. A 10 mL volume of 0.01 M NBS was found adequate for quantitative oxidation of ZDV in the range investigated. The linearity between the volume at the endpoint and drug amount is apparent from the calculated correlation coefficient of –0.9852 suggesting that the reaction between ZDV and NBS proceeds stiochiometrically in the ratio 1:2.

The proposed spectrophotometric methods are indirect and are based on the determination of the residual NBS after allowing the reaction between ZDV and a measured amount of NBS to be complete. The residual NBS was determined by reacting it with a fixed amount of either methyl orange or indigo carmine dye. The methods make use of bleaching action of NBS on the dyes, the decolouration being caused by the oxidative destruction of the dyes.
ZDV + Known excess of NBS → Reaction Product + Unreacted NBS
Unreacted NBS + Methyl orange → Absorbance measured at 520 nm (method A)
Unreacted NBS + Indigo Carmine → Absorbance measured at 610 nm (method B)

ZDV when added in increasing concentrations to a fixed concentrations of NBS, consumes the latter proportionally and there occurs a concomitant fall in the concentration of NBS. When a fixed concentration of dye is added to decreasing concentrations of NBS, a concomitant increase in the concentration of dye results. Consequently, a proportional increase in the absorbance at the respective $\lambda_{\text{max}}$ is observed with increasing concentration of ZDV (Fig. 2.2.1 and 2.2.3).

![Beer's law curve for method A](image1)

**Fig. 2.2.1.** Beer’s law curve for method A

![Beer's law curve for method B](image2)

**Fig. 2.2.2.** Beer’s law curve for method B

Preliminary experiments were performed to fix the upper concentrations of the dyes that could be determined spectrophotometrically, and these were found to be 5 and 20 $\mu$g mL$^{-1}$ for methyl orange and indigo carmine respectively. A NBS
concentration of 8.0 µg mL\(^{-1}\) was found to bleach the red colour due to 5 µg mL\(^{-1}\) methyl orange whereas 34.0 µg mL\(^{-1}\) NBS was required to destroy the blue colour due to 20 µg mL\(^{-1}\) indigocarmine. Hence, different concentrations of ZDV were reacted with 1 mL each of 80 µg mL\(^{-1}\) NBS in method A and 340 µg mL\(^{-1}\) NBS in method B before estimating the unreacted NBS employing methyl orange and indigocarmine, respectively. This facilitated fixing the concentration ranges over which ZDV could be determined by each method. For both steps, i.e., the reaction between ZDV and NBS, and the determination of the latter by reacting with the dye, HCl medium was found to be ideally suited. The absorbance of the dye was not affected in 0.1 to 0.3 M HCl concentrations. However, 1 mL of 1M HCl was selected for oxidation of drug in both methods and the same quantity of acid was maintained for bleaching step. The oxidation reaction was found to be complete in 5 min and contact times upto 30 min had no effect on the absorbance of dyes. The absorbance of either dyes solution even in the presence of reaction product was found to be stable for several days.

### 2.2.4.2. Method Validation

**Linearity Range and Sensitivity**

Linearity was observed over the concentration ranges 0.5 -6.0 and 2.5 -20 µg mL\(^{-1}\) ZDV for method A and method B, respectively, confirming the adherence of the system to Beer’s law. Absorbance and concentration data were subjected to linear least-squares regression analysis to calculate the calibration equation and correlation coefficients (Table. 2.2.1).

The limits of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines [80] are also presented in Table 2.2.1 and reveal the very high sensitivity of the spectrophotometric methods.
Table 2.2.1- Analytical and regression parameters of spectrophotometric methods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method A</th>
<th>Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$, nm</td>
<td>520</td>
<td>610</td>
</tr>
<tr>
<td>Beer’s law limits, $\mu g \text{ mL}^{-1}$</td>
<td>0.5 – 6.0</td>
<td>2.5 – 20.0</td>
</tr>
<tr>
<td>Molar absorptivity, L mol$^{-1}$ cm$^{-1}$</td>
<td>$2.7 \times 10^4$</td>
<td>$8.2 \times 10^3$</td>
</tr>
<tr>
<td>Sandell sensitivity, $\mu g \text{ cm}^2$</td>
<td>0.0098</td>
<td>0.033</td>
</tr>
<tr>
<td>Limit of detection, $\mu g \text{ mL}^{-1}$</td>
<td>0.09</td>
<td>0.25</td>
</tr>
<tr>
<td>Limit of quantification, $\mu g \text{ mL}^{-1}$</td>
<td>0.27</td>
<td>0.74</td>
</tr>
<tr>
<td>Regression equation, $Y^*$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept ($a$)</td>
<td>-0.020</td>
<td>-0.015</td>
</tr>
<tr>
<td>Slope ($b$)</td>
<td>0.1086</td>
<td>0.032</td>
</tr>
<tr>
<td>Correlation coefficient, ($r$)</td>
<td>0.9993</td>
<td>0.9996</td>
</tr>
<tr>
<td>$S_a$</td>
<td>0.008</td>
<td>0.007</td>
</tr>
<tr>
<td>$S_b$</td>
<td>0.002</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* $Y = a+bX$, where $Y$ is the absorbance and $X$ concentration in $\mu g \text{ mL}^{-1}$

$S_a$ = Standard deviation of intercept.

$S_b$ = Standard deviation of slope.

**Intra-day and inter-day accuracy and precision**

The precision of the methods was evaluated in terms of intermediate precision (intra-day and inter-day). Three different concentrations of ZDV (within the Beer’s law range) were analysed in seven replicates during the same day (intra-day precision) and five consecutive days (inter-day precision) by preparing all solutions afresh each day. The RSD values of intra-day and inter-day precision studies for both methods showed that the precision was satisfactory (Table 2.2.2).

The accuracy was evaluated as percentage relative error between the measured mean concentrations and added concentrations of ZDV (Bias, %). The results obtained for three different concentrations are shown in Table 2.2.2, from which it is clear that the accuracy is fairly good in both the methods.
Table 2.2.2. Intra-day and inter-day accuracy and precision studies

<table>
<thead>
<tr>
<th>Method*</th>
<th>ZDV taken</th>
<th>Intra-day accuracy and precision&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Inter-day accuracy and precision&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ZDV taken</td>
<td>ZDV found</td>
<td>RE, %</td>
</tr>
<tr>
<td>Titrimetry</td>
<td>5.0</td>
<td>5.09</td>
<td>1.80</td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>8.77</td>
<td>2.56</td>
</tr>
<tr>
<td></td>
<td>13.0</td>
<td>13.20</td>
<td>1.54</td>
</tr>
<tr>
<td>Spectrophotometric method A</td>
<td>2.0</td>
<td>1.98</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>2.89</td>
<td>3.67</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>3.98</td>
<td>0.50</td>
</tr>
<tr>
<td>Spectrophotometric method B</td>
<td>5.0</td>
<td>5.06</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>9.85</td>
<td>1.50</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>14.95</td>
<td>0.33</td>
</tr>
</tbody>
</table>

RE. relative error; RSD. relative standard deviation.
*In titrimetry, ZDV taken/ found are in mg, while in the spectrophotometric methods, the quantities are in µg mL<sup>-1</sup>
<sup>a</sup>n=7; <sup>b</sup>n=5.

Robustness and ruggedness

To check the robustness of the methods, acid concentration and reaction time were marginally altered and the intermediate precision was determined employing a single concentration of ZDV. The percent RSD for three different acid concentrations was < 3.5 % and the same for three reaction times was < 4.0 % indicating that the methods are robust enough for routine analysis. The ruggedness of the methods was assessed by calculating the RSD for results obtained by performing the analysis using three different instruments and by three different persons. The inter-instrumental RSD values ranged from 2.8-3.9 % whereas the inter-personal RSD values varied from 1.9-3.5% for three concentrations of ZDV employed for accuracy and precision. The study suggested that the methods were robust as well as rugged.

Selectivity

To evaluate the usefulness of the methods, the effect of excipient and additives, which often accompany ZDV in tablet/capsule, was studied. A synthetic mixture with the composition: ZDV (100 mg); NaCl (200 mg); talc (50 mg); starch (150 mg); lactose (200 mg); titanium dioxide (50 mg); sodium alginate (50 mg) and
magnesium stearate (25 mg), was prepared and the active component (ZDV) was extracted with water as described under procedure for tablets and analysed as recommended procedure. The percentage recoveries (n=5) of ZDV was found to be 98.8±3.3 for titrimetry, 99.5±1.6 for spectrophotometric method A and 99.1±2.0 for spectrophotometric method B, indicating high selectivity for determining the studied drug in formulations.

Application

The validity of the methods was checked by applying them to assay in two brands of capsules and tablets. Table 2.2.3 gives the results of assay and reveals that there is close agreement between the results obtained by the proposed methods and the label claim. The results were also compared statistically with those obtained by a reference method [5] by applying Student’s t-test for accuracy and F-test for precision. At the 95% confidence level, the calculated t- and F-values did not exceed the tabulated values, (t = 2.77 and F = 6.39) suggesting that the proposed methods are as accurate and precise as the reference method.

Table 2.2.3- Results of determination of zidovudine in formulations and statistical comparison with the reference method

<table>
<thead>
<tr>
<th>Formulation Brand name²</th>
<th>Nominal amount, mg</th>
<th>% found± SD</th>
<th>Reference method</th>
<th>Titrimetry</th>
<th>Spectrophotometric method A</th>
<th>Spectrophotometric method B</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIRO-Z²</td>
<td>100</td>
<td>101.5±1.14</td>
<td>100.8±0.54</td>
<td>102.1±1.12</td>
<td>102.9±1.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>t=1.32</td>
<td>t=0.84</td>
<td>t=1.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F=4.46</td>
<td>F=1.04</td>
<td>F=1.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>100.6±0.85</td>
<td>101.8±1.24</td>
<td>101.3±1.42</td>
<td>99.76±1.02</td>
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</tr>
<tr>
<td></td>
<td></td>
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<td>t=1.81</td>
<td>t=0.97</td>
<td>t=1.42</td>
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</tr>
<tr>
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<td></td>
<td></td>
<td>F=2.13</td>
<td>F=2.80</td>
<td>F=1.44</td>
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</tr>
<tr>
<td></td>
<td>100</td>
<td>97.02±0.56</td>
<td>97.2±0.45</td>
<td>98.1±1.36</td>
<td>98.16±1.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>t=0.56</td>
<td>t=1.78</td>
<td>t=2.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F=1.55</td>
<td>F=5.90</td>
<td>F=4.30</td>
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<tr>
<td></td>
<td>300</td>
<td>99.66±0.82</td>
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<td>101.3±1.32</td>
<td>100.8±1.33</td>
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<tr>
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<td></td>
<td></td>
<td>t=1.33</td>
<td>t=2.42</td>
<td>t=1.68</td>
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<td></td>
<td></td>
<td></td>
<td>F=3.18</td>
<td>F=2.59</td>
<td>F=2.63</td>
<td></td>
</tr>
</tbody>
</table>

*Mean value of five determinations
#Marketed by: a. Ranbaxy Ltd. India.; b. Genix Ltd.
Tabulated t-value at 95% confidence level is 2.77
Tabulated F-value at 95% confidence level is 6.39.
Recovery Study

The accuracy and validity of the proposed methods were further ascertained by performing recovery experiments. Pre-analysed tablet/capsule powder was spiked with pure ZDV at three different levels and the total was found by the proposed methods. Each determination was repeated three times. The recovery (Table 2.2.4.) of pure drug added was quantitative and revealed that co-formulated substances did not interfere in the determination.

Table 2.2.4- Results of recovery experiments by standard addition method

<table>
<thead>
<tr>
<th>Formulation Studied</th>
<th>Titrimetry</th>
<th>Spectrophotometric method A</th>
<th>Spectrophotometric method B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ZDV in tablet, mg</td>
<td>Pure ZDV added, mg</td>
<td>Total found, mg</td>
</tr>
<tr>
<td>VIRO-Z 100</td>
<td>4.99</td>
<td>2.5</td>
<td>7.55</td>
</tr>
<tr>
<td></td>
<td>4.99</td>
<td>5.0</td>
<td>10.07</td>
</tr>
<tr>
<td></td>
<td>4.99</td>
<td>7.5</td>
<td>12.44</td>
</tr>
<tr>
<td>ZIDO-H 300</td>
<td>5.01</td>
<td>2.5</td>
<td>7.56</td>
</tr>
<tr>
<td></td>
<td>5.01</td>
<td>5.0</td>
<td>9.91</td>
</tr>
<tr>
<td></td>
<td>5.01</td>
<td>7.5</td>
<td>12.55</td>
</tr>
</tbody>
</table>

*Mean value of three determinations.
SECTION 2.3

SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF ZIDOVUDINE USING N-BROMOSUCCINIMIDE, METOL AND SULPHANILIC ACID*

2.3.1.0. INTRODUCTION

Important features of N-bromosuccinimide (NBS) as an oxidimetric reagent were described in Section 2.2.1.0 and applications of NBS in the titrimetric and the spectrophotometric determination of a variety of pharmaceuticals were also reviewed in Section 2.2.1.0.

Indirect spectrophotometric methods often alleviate the problems of critical working conditions, instability of the coloured species and interference from concomitant substances encountered in direct spectrophotometric methods. As a result, in recent years, indirect spectrophotometry has found wide applications in various fields of analysis including pharmaceuticals analysis [136-152].

One of the sensitive methods of general applicability for the determination of primary aromatic amines involves the formation of purple-red colour when primary aromatic amines are made to react with metol (p-N-methylamino phenol) and an oxidizing agent which may be dichromate [153,154], N-bromosuccinimide[155], 2-iodyl benzoate [156] or iodate, vanadate, iron(III), hexacyanoferrate(III), peroxy disulphate or chloramine-T[157]. The purple-red colour is believed to be a charge transfer complex formed between the oxidation product generated insitu from metol[154] and the primary aromatic amine studied. The utility of this reaction has been extended to the determination of amino acids using CAT or sodium hypochlorite[158]. Several other amino compounds such as aminophenols, thiols and antioxidants have been quantitated with metol and oxidants like metaperiodate [159], iron(III)[160] and permanganate [161], respectively.

Reaction of metol with oxidant has been applied to the assay of many pharmaceutically important compounds containing amino group like, pindolol and captopril[162], antiameobics and anthelmintics[163], muzolimine[164], pencillins G and V [165] and benzyl and phenoxy methyl pencillins[166], the oxidant being Cr(VI).

*This work has been published in E-J. Chem., 2007, 4(2), 173-179
Periodate-metol combination has been used for the determination of thiazide diuretics [167], sulphanmethoxy pyridazine [168] and sulphadoxine sulphaline [169]. Isoniazid [170] has been determined with metol and vanadate, hexacyanoferrate (III) or iron (III). Metol in combination with iodine and with permanganate has been applied to the assay of antitubercular drugs [171] and acebutalol hydrochloride and nicoumalone [172], respectively.

In all these cases the intensity of the colour produced was found to be proportional to the concentration of the primary aryl amines [153-157] or amino compounds [158-172].

The reaction of metol with primary aromatic amine in the presence of an oxidizing agent can be used for the spectrophotometric determination of the latter, thus facilitating the indirect assay of oxidisable substances. In this approach, the drugs are treated with a known excess of the oxidant and, after the reaction, the unreacted oxidant is determined by reacting with metol and a primary aromatic amine such as sulphanilic acid. The oxidant-metol-primary aromatic amine combination has been used for the indirect spectrophotometric determination of a considerable number of oxidisable medicinal compounds such as 2-thiouracil [173], methotrexate [174], rutin [175], timolol [176] and benzimidazole anthelmintics [177].

Several pharmaceutically important primary aryl amines have been determined spectrophotometrically with metol using N-bromosuccinimide(NBS) as the oxidizing agent [178]. NBS-metol-primary arylamine combination can also be used for the determination of the oxidant, thereby permitting the indirect assay of many oxidisable substances including drugs. In this procedure, the drug is oxidized with a known excess of NBS and, after the reaction, the unreacted NBS is reacted with metol and primary arylamine, and the purple colour formed is measured and correlated to the drug concentration. Many pharmaceuticals like benzimidazole anthelmintics[179], methotrixate [180], rutin [181], timolol [182], thiouracil [183], vitamin-C [184], sulphonamides [185], propranolol hydrochloride [186], nifurtimox [187], paracetamol [188], diltiazem hydrochloride [189], cyproheptadine hydrochloride [190], prozosin hydrochloride [191], omeprazole [192], thiazide diuretics [193] and thiols [194] have been estimated by this approach using NBS as the oxidant.
Based on the above reaction schemes, the author used NBS successfully for the spectrophotometric determination of ZDV. The method is based on the oxidation of ZDV by a known excess of N-bromosuccinimide (NBS), in buffer medium of pH 1.5, followed by the estimation of unreacted amount of oxidant with metol and sulphanilic acid. The reacted oxidant corresponds to the amount ZDV. The details of the method are presented in this Section, 2.3.

2.3.2.0. EXPERIMENTAL

2.3.2.1. Apparatus

The instrument used is the same as the one described in section 2.1.2.1.

2.3.2.2. Reagents and Standards

All used chemicals were of analytical reagent grade of purity and all solutions were prepared in distilled water.

**N- bromosuccinimide, NBS (1000 µg mL⁻¹).** Preparation of NBS (~0.01M) and its standardization are described in Section 2.2.2.2. The solution was later diluted to obtain 1000 µg mL⁻¹ NBS.

**Metol (0.2%).** Prepared by dissolving 200 mg of chemical (s.d. Fine Chem., India) in 100 mL water.

**Sulphanilic acid (0.2%).** Prepared by dissolving 200 mg of chemical (Ranbaxy Fine Chem., India) in 100 mL water.

**Buffer pH 1.5.** Prepared by mixing equal volumes of N-HCl and N-sodium acetate and adjusting to pH 1.5 by varying either of them.

**Standard solution of zidovudine.** Prepared as described in section 2.1.2.2. The stock solution was diluted appropriately with water to get a working concentration of 250 µg mL⁻¹. The standard solutions were kept in amber coloured bottle and stored in a refrigerator when not in use.

2.3.3. PROCEDURES

Varying aliquots (0.2----3.0 mL) of a standard 250 µg mL⁻¹ ZDV solution were transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was brought to 3 mL by adding water. To each flask were added 1 mL of buffer pH 1.5 and 1.0 mL of NBS (1000 µg mL⁻¹) the last being added by
means of a micro burette. The content was mixed well and the flasks were kept aside for 10 min with intermittent shaking. Then, 1mL of metol and after 1 min, 1 mL of sulphanilic acid were added to each flask, the volume was diluted to the mark with water, mixed well and absorbance measured against distilled water at 530 nm during the stability period of 10-50 min.

Calibration graph was prepared by plotting decreasing values of absorbances against drug concentration. The concentration of the unknown was read from the calibration graph or computed from the regression equation derived using the Beer’s law data.

**Pharmaceutical preparations**

Prepared as described in section 2.1.3. The filtrate (1000 µg mL⁻¹ ZDV) was appropriately diluted with water to get a working concentration of 250 µg mL⁻¹ ZDV and analysed by taking convenient aliquots.

### 2.3.4. RESULTS AND DISCUSSION

#### 2.3.4.1. Method development

The method is based on the oxidation of the drug by a known excess of NBS in buffer medium and subsequent determination of the unreacted NBS by interacting with metol and the primary aromatic amine, sulphanilic acid.

ZDV, when added in increasing concentrations to a fixed concentration of NBS, consumes NBS and consequently there will be a concomitant fall in the NBS concentration. This is observed as a proportional decrease in the absorbance of the reaction mixture on increasing the concentration of drug.

It is probable that the p-N-methylbenzoquinone monoamine \[195,196\] formed *insitu* from the metol-NBS reaction, being a good electron acceptor, forms a charge transfer complex with the amine as electron donor. The probable reaction scheme is shown in Fig.2.3.1.
The first step in this study was to fix the upper limit of NBS, which was found by treating different concentrations of NBS with metol, and suphanilic acid under the conditions described in the general procedure. The study showed that Beer’s law is obeyed up to 100 µg mL⁻¹ NBS. Hence, different concentration of ZDV was reacted with 1 mL of 1000 µg mL⁻¹ NBS and the unreacted NBS was determined by following the general procedure.

Fig. 2.3.2. Linearity curve

In this study, two blanks were prepared. The reagent blank, which contained optimum concentrations of all reactant except drug, gave maximum absorbance. The
other blank was prepared in the absence of NBS and drug to determine the contribution of other reactants to the absorbance of the system. Since the absorbance of the second blank was negligible, the absorbance of the developed colour was measured against water.

The reaction conditions were established by varying of one parameter at a time. One mL of buffer pH 1.5 was found optimal, when varied from 1 to 3 mL in a total volume of 10 mL. The maximum colour intensity developed in 10 min and was stable upto 50 min thereafter.

Incomplete colour development was observed when the order of addition was changed. Therefore order of addition should be as in the general procedure. Any delay in the addition of sulphanilic acid also results in low and variable absorbance values. Addition of sulphanilic acid should not be delayed beyond 10 min after adding metol.

2.3.4.2. Method Validation

Linearity and Sensitivity

A linear correlation was found between absorbance at $\lambda_{\text{max}}$ and concentration of ZDV in the range given in Table 2.3.1. The graph (Fig. 2.3.2) is described by the regression equation: $Y = a + bX$

(Where $Y$ = absorbance of 1-cm layer of solution; $a$ = intercept; $b$ = slope and $X$ = concentration in $\mu$g mL$^{-1}$). Regression analysis of the Beer’s law data using the method of least squares was made to evaluate the slope ($b$), intercept ($a$) and correlation coefficient($r$) for each system and the values are presented in Table 2.3.1. The optical characteristics such as Beer’s law limits, molar absorptivity and Sandell sensitivity value, LOD, LOQ of method are also given in Table 2.3.1.
Table 2.3.1. Analytical and regression parameters of proposed method.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$, nm</td>
<td>530</td>
</tr>
<tr>
<td>Beer’s law limits, $\mu \text{g mL}^{-1}$</td>
<td>5.0 – 75.0</td>
</tr>
<tr>
<td>Molar absorptivity, L $\text{mol}^{-1} \text{cm}^{-1}$</td>
<td>$5.1 \times 10^3$</td>
</tr>
<tr>
<td>Sandell sensitivity, $\mu \text{g cm}^{-2}$</td>
<td>0.0524</td>
</tr>
<tr>
<td>Limit of detection, $\mu \text{g mL}^{-1}$</td>
<td>0.90</td>
</tr>
<tr>
<td>Limit of quantification, $\mu \text{g mL}^{-1}$</td>
<td>2.72</td>
</tr>
<tr>
<td>Regression equation, $Y$</td>
<td></td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.8113</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>-0.011</td>
</tr>
<tr>
<td>Correlation coefficient, (r)</td>
<td>-0.9993</td>
</tr>
<tr>
<td>$S_a$</td>
<td>0.1929</td>
</tr>
<tr>
<td>$S_b$</td>
<td>0.009</td>
</tr>
</tbody>
</table>

*Y = a+bX, where Y is the absorbance and X concentration in $\mu \text{g mL}^{-1}$.

$S_a$ = Standard deviation of intercept.
$S_b$ = Standard deviation of slope.

Intra-day and inter-day accuracy and precision

The precision of the method was evaluated in terms of intermediate precision (intra-day and inter-day). Three different concentrations of ZDV (within the Beer’s law range) were analysed in seven replicates during the same day (intra-day precision) and five consecutive days (inter-day precision) by preparing all solutions afresh each day. The RSD values of intra-day and inter-day precision studies for both methods showed that the precision was satisfactory (Table 2.3.2).

The accuracy was evaluated as percentage relative error between the measured mean concentrations and added concentrations of ZDV (Bias, %). The results obtained for three different concentrations are shown in Table 2.3.2, from which it is clear that the accuracy is fairly good in both the methods.
Table 2.3.2. Intra-day and inter-day accuracy and precision studies

<table>
<thead>
<tr>
<th>Method*</th>
<th>ZDV taken</th>
<th>Intra-day accuracy and precision&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Inter-day accuracy and precision&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ZDV found</td>
<td>RE, %</td>
<td>RSD, %</td>
</tr>
<tr>
<td>Spectrophotometric method</td>
<td>12.5</td>
<td>12.35</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td>37.5</td>
<td>37.07</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>62.5</td>
<td>61.67</td>
<td>1.33</td>
</tr>
</tbody>
</table>

RE, relative error; RSD, relative standard deviation.
<sup>a</sup> The quantities are in µg mL<sup>-1</sup>
<sup>b</sup> n=7; n=5.

Robustness and ruggedness

To evaluate the method robustness, only the reaction time was altered. The ruggedness of the method was assessed by calculating the RSD for results obtained by performing the analysis using three different instruments and by three different persons. The inter-instrumental RSD values ranged from 2.8-5.5 % whereas the inter-personal RSD values varied from 2.8-3.5% for three concentrations of ZDV employed for accuracy and precision. The study suggested that the method was robust as well as rugged.

Selectivity

To evaluate the usefulness of the method, the effect of excipient and additives, which often accompany ZDV in tablet/capsule, was studied. A synthetic mixture with the composition: ZDV (100 mg); NaCl (200 mg); talc (50 mg); starch (150 mg); lactose (200 mg); titanium dioxide (50 mg); sodium alginate (50 mg) and magnesium stearate (25 mg), was prepared and the active component (ZDV) was extracted with water as described under procedure for tablets and analysed as recommended procedure. The percentage recoveries (n=5) of ZDV was found to be 99.8+1.3, indicating high selectivity for determining the studied drug in formulations.

Application to formulations

Table 2.3.3 gives the results of assay and reveals that there is close agreement between the results obtained by the proposed method and the label claim. The results were also compared statistically with those obtained by a reference method [5], by applying Student’s t-test for accuracy and F-test for precision. At the 95% confidence level, the calculated t- and F-values did not exceed the tabulated values (t = 2.77 and F
s = 6.39) suggesting that the proposed method is as accurate and precise as the reference method.

Table 2.3.3. Results of determination of zidovudine in formulations and statistical comparison with the reference method.

<table>
<thead>
<tr>
<th>Tablet/ Capsule Brand name*</th>
<th>Nominal amount, mg</th>
<th>% Found* ± SD</th>
<th>Reference method</th>
<th>Proposed method</th>
<th>t value</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean value of five determinations. #Marketed by: a. Ranbaxy Ltd. India; b. Genix Ltd. Tabulated t-value at 95% confidence level is 2.77 Tabulated F-value at 95% confidence level is 6.39</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIRO-Z a (Tablets)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100.8±0.58.</td>
<td>99.8±0.82</td>
<td>2.26</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>99.50±0.36.</td>
<td>99.1±0.45</td>
<td>1.56</td>
<td>1.56</td>
<td></td>
</tr>
<tr>
<td>ZIDO-H b (Capsules)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>101.2±0.51.</td>
<td>100.8±0.40</td>
<td>1.39</td>
<td>1.63</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>100.5±0.62.</td>
<td>101.3±0.55</td>
<td>2.16</td>
<td>1.27</td>
<td></td>
</tr>
</tbody>
</table>

Recovery Study

The accuracy and validity of the proposed method was further ascertained by performing recovery experiments (Table 2.3.4). Pre-analyzed tablet/capsule powder was spiked with pure ZDV at three different levels and the total was found by the proposed method. Each determination was repeated three times. The recovery of pure drug added was quantitative and revealed that co-formulated substances did not interfere in the determination.

Table 2.3.4- Results of recovery experiments

<table>
<thead>
<tr>
<th>Formulation studied</th>
<th>ZDV in formulation, µg mL⁻¹</th>
<th>ZDV added, µg mL⁻¹</th>
<th>Total found µg mL⁻¹</th>
<th>Pure ZDV recovered* %</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIRO-Z 100 (Tablets)</td>
<td>9.98</td>
<td>10</td>
<td>20.01</td>
<td>100.3</td>
</tr>
<tr>
<td></td>
<td>9.98</td>
<td>30</td>
<td>39.92</td>
<td>99.8</td>
</tr>
<tr>
<td></td>
<td>9.98</td>
<td>50</td>
<td>60.53</td>
<td>101.1</td>
</tr>
<tr>
<td></td>
<td>10.1</td>
<td>10</td>
<td>20.07</td>
<td>99.7</td>
</tr>
<tr>
<td>ZIDO-H 300 (Capsules)</td>
<td>10.1</td>
<td>30</td>
<td>39.59</td>
<td>98.3</td>
</tr>
<tr>
<td></td>
<td>10.1</td>
<td>50</td>
<td>60.30</td>
<td>100.4</td>
</tr>
</tbody>
</table>

*Mean value of three determinations
SECTION 2.4

TITRIMETRIC AND SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF ZIDOVUDINE USING CHLORAMINE-T AND TWO DYSES METHYL ORANGE AND INDIGO CARMINE AS REAGENTS*

2.4.1.0. INTRODUCTION

Chloramine-T (abbreviated as CAT), the sodium salt of N-chloro-p-toulene sulphonamide, is a prominent member of aromatic sulphoxyl haloamines. It has the following structure:

\[
\begin{align*}
\text{CH}_3 & \\
\text{SO}_2 & \\
\text{Na} & \\
\text{Cl} & \\
3\text{H}_2\text{O} & 
\end{align*}
\]

CAT was first prepared by Chattaway [197]. It is a versatile oxidizing agent with a redox potential of 1.52 V in 1.0 N \( \text{H}_2\text{SO}_4 \). The titration with CAT is in some cases preferred to the more costly iodometric determinations. It has the advantage that it may be used in acid or alkaline solution [198] and is said to be more stable than hypochlorite. Aqueous CAT solutions are very stable. If stored in dark bottles, they do not change their true titre for several months; boiling for several hours likewise does not change their true titre [199].

The above properties make CAT a useful analytical reagent. Noll [200] was the first to propose the use of CAT as an oxidimetric titrant. More than 200 papers have been published since 1924 on the use of CAT in chemical analysis. The behaviour of CAT as a titrimetric reagent has critically been examined by Bishop and Jennings [201] and by Jennings [202]. A large number of substances, both organic and inorganic, have been estimated using CAT as an oxidimetric titrant with visual, potentiometric and amperometric end point detection [203, 204]. The applications of CAT as an oxidimetric titrant have been reviewed by Rangaswamy [205] and Venkatachalapathy [206].

*This work has been published in Indian J. Chem. Technol. 2007, 14, 200-203
The versatility of CAT as an oxidimetric titrant can be gauged by innumerable organic and inorganic substances that have been determined \[203-206\]. In addition, many substances of pharmaceutical importance have also been estimated using CAT as the oxidimetric reagent followed by iodometric back titration of the residual oxidant. Such substances include caffeine \[207\], nifenazone \[208\], papaverine \[209\], ironicotanic hydrazide \[210\], penicillins \[211\], dopamine hydrochloride \[212\], captopril \[213\], ranitidine \[214\], albendazole \[215\], famotidine \[216\] and phenothiazines \[217\].

Several substances of pharmaceutical interest bromherine \[218\], piroxicam \[219\], tetracyclines \[220\] such as metacycline and rifampicin \[221\], carbimazole and thiopentone \[222\], benzthiazide and hydroflurothiazide \[223\], antihistamines \[224\], omeprazole \[225\], diuretics \[226\], antibiotics and anthelmentics \[227\], cephalaxin \[228\] and thiouracil \[229\], propanolol \[230\], atenolol \[231\], albendazole\[232,233\], famotidine\[234\] have been determined by indirect spectrophotometry using CAT as a reagent based on different reaction schemes.

Certain organic dyes are destroyed by CAT in acid medium, and hence these dyes have served as redox indicators in titrations involving CAT as the oxidant. The dual properties (oxidizing and bleaching) of CAT have also been exploited for the assay of some oxidisable pharmaceuticals \[230-234\].

From the review of the literature on the assay methods for ZDV using various techniques presented in Section 2.0.2 it is obvious that ZDV has not been determined by chloramine-T using titrimetric and spectrophotometric techniques. In this work, three new methods using titrimetry and spectrophotometry are described for the assay of zidovudine (ZDV) in bulk drug and in dosage forms using chloramine-T (CAT) and two dyes, methyl orange and indigocarmine, as reagents. Titrimetry involves treating of ZDV with a measured excess of CAT in hydrochloric acid medium, and after the oxidation of ZDV is judged to be complete, the unreacted oxidant is determined iodometrically. Spectrophotometric methods entail the addition of a known excess of CAT to ZDV in hydrochloric acid medium followed by determination of residual oxidant by reacting with a fixed amount of either methyl orange and measuring the absorbance at 520 nm (Method A) or indigo carmine and measuring the absorbance at 610 nm (Method B). The details of the assay methods are presented in this Section (2.4).
2.4.2.0. EXPERIMENTAL

2.4.2.1. Apparatus

The instrument is the same that was described in section 2.1.2.1.

2.4.2.2. Reagents and Standards

All chemicals used were of analytical reagent grade and distilled water was used to prepare all solutions.

**Chloramine-T solution (5 m M):** Prepared by dissolving about 1.4 g of the chemical (Qualigens fine chem., Glaxo India Ltd., Mumbai) in water and diluting to 1 litre, and used in titrimetry after standardization[235]. For spectrophotometric investigation, the above solution was diluted appropriately with water to get 55 and 200 µg mL⁻¹ concentrations for spectrophotometric method A and method B, respectively. Preparation of methyl orange(50 µg mL⁻¹), indigo carmine (200 µg mL⁻¹), potassium iodide (10 %), starch indicator (1 %) ; hydrochloric acid (2 M) and sodium thiosulphate solution (0.01 M) is described in section 2.2.2.2.

**Standard drug solution:** Prepared as described in section 2.1.2.2. This stock solution, 1 mg mL⁻¹ was used for titrimetry and diluted with water to get working concentrations of 10 and 50 µg mL⁻¹ ZDV for spectrophotometric method A and method B, respectively.

2.4.3. PROCEDURES

2.4.3.1. Titrimetry

A 10 mL aliquot of pure drug solution equivalent to 3.0-10 mg of ZDV was measured accurately and transferred into a 100 mL titration flask. Five mL 2 M hydrochloric acid followed by 10 mL of 5 m M chloramine-T solution were added and kept aside for 10 min with occasional swirling. Then, 5 mL of 10 % potassium iodide solution were added to the flask and the liberated iodine was titrated with 0.01 M sodium thiosulphate to a starch end point. A blank titration was run under same conditions. The amount of the drug present in the measured aliquot was calculated from the volume of chloramine-T that has reacted with the drug.

2.4.3.2. Spectrophotometric method using methyl orange (Method A)

Aliquots of pure ZDV solution (0.5 to 5.0 mL; 10 µg mL⁻¹) were transferred into a series of 10 mL calibrated flasks and the total volume was adjusted to 5.0 mL with water. To each flask were added 1 mL each of 2 M hydrochloric acid and chloramine-T solution (55 µg mL⁻¹). The contents were mixed well and the flasks
were set aside for 15 min with occasional shaking. Finally, 1 mL of 50 µg mL\(^{-1}\) methyl orange solution was added to each flask, diluted to the mark with water and the absorbance of solution was measured at 520 nm against reagent blank after 10 min.

2.4.3.3. Spectrophotometry with indigo carmine (Method B)

Varying aliquots (0.5-4.0 mL) of standard 50 µg mL\(^{-1}\) ZDV solution were measured accurately and delivered into a series of 10 mL calibrated flasks and the total volume was brought to 4.0 mL with water. To each flask were added 1 mL each of 2 M hydrochloric acid and 200 µg mL\(^{-1}\) chloramine-T solutions successively; the flasks were let stand for 15 min with occasional shaking. Then, 1 mL of 200 µg mL\(^{-1}\) indigo carmine solution was added to each flask, the volume was adjusted to the mark with water and mixed well. The absorbance of each solution was measured at 610 nm against a reagent blank after 10 min. In either spectrophotometric method, the concentration of the unknown was read from the calibration graph or computed from the regression equation derived from the Beer’s law data.

**Assay procedure for formulations**

Solutions of dosage forms were prepared as described in section 2.1.3 and a suitable aliquot of the subsequent portion (1 mg mL\(^{-1}\) ZDV) was taken for assay by titrimetric procedures. The filtrate was diluted appropriately to get 10 and 50 µg mL\(^{-1}\) concentrations for analysis by spectrophotometric method A and method B, respectively.

2.4.4. RESULTS AND DISCUSSION

The proposed methods are based on the oxidation of ZDV by CAT in HCl medium and the reaction is followed by titrimetry and spectrophotometry for quantization purposes. The possible reaction scheme is presented below:

**Titrimetry**

\[ \text{ZDV} + \text{Known excess of CAT} \xrightarrow{\text{H}^+} \text{Reaction product} + \text{Unreacted CAT determined iodometrically} \]

**Spectrophotometry**

\[ \text{ZDV} + \text{Known excess of CAT} \xrightarrow{\text{H}^+} \text{Reaction Product} + \text{Unreacted CAT} \]

\[ \text{Unreacted CAT} + \text{Methyl orange} \xrightarrow{} \text{Absorbance measured at 520 nm (method A)} \]

\[ \text{Unreacted CAT} + \text{Indigo Carmine} \xrightarrow{} \text{Absorbance measured at 610 nm (method B)} \]
2.4.4.1. Method development

**Titrimetry:** The reaction stoichiometry was found to be 1:1 (ZDV: CAT) and was unaffected in the presence of 5 to 10 mL of 2 M HCl in a total volume of 25 mL and 5 mL of 2 M HCl was chosen as the optimum volume. The oxidation reaction was found to be complete in 10 min and contact time up to 30 min had no effect on the stoichiometry or the results. A 10 mL volume of 5 mM CAT solution was found adequate for the quantitative oxidation of ZDV in the range studied, 3-10 mg.

**Spectrophotometry:** In the proposed spectrophotometric methods, the ability of chloramine-T to effect oxidation of ZDV and irreversibly destroy methyl orange or indigo carmine to colourless product in acid medium has been used. ZDV when added in increasing concentrations to a fixed concentrations of CAT, consumes the latter proportionally and there occurs a concomitant fall in the concentration of CAT. When a fixed concentration of dye is added to decreasing concentration of CAT, a concomitant increase in the concentration of dye results. Consequently, a proportional increase in the absorbance at the respective $\lambda_{max}$ is observed with increasing concentration of ZDV (Fig. 2.4.1 and 2.4.2).

![Fig. 2.4.1. Beer’s law curve for method A](image1)

![Fig. 2.4.2. Beer’s law curve for method B](image2)
Preliminary experiments were performed to fix the upper limits of the dyes that could be determined spectrophotometrically, and these were found to be 5 and 20 \( \mu g \text{ mL}^{-1} \) for methyl orange and indigo carmine, respectively. A chloramine-T concentration of 5.5 \( \mu g \text{ mL}^{-1} \) was found to irreversibly destroy the red colour of 5 \( \mu g \text{ mL}^{-1} \) methyl orange whereas 20.0 \( \mu g \text{ mL}^{-1} \) chloramine-T was required to bleach the blue colour due to 20 \( \mu g \text{ mL}^{-1} \) indigo carmine. Hence, different amounts of ZDV were reacted with 1 mL of 55 \( \mu g \text{ mL}^{-1} \) chloramine-T in method A and 1mL of 200 \( \mu g \text{ mL}^{-1} \) chloramine-T in method B followed by determination of the residual oxidant as described under the respective procedures.

2.4.4.2. Method Validation

Linearity and sensitivity

A linear correlation was found between absorbance at \( \lambda_{\text{max}} \) and concentration of ZDV. The graphs showed negligible intercept and are described by the equation:

\[
Y = a + bX
\]

(Where \( Y \) = absorbance of 1-cm layer of solution; \( a \) = intercept; \( b \) = slope and \( X \) = concentration in \( \mu g \text{ mL}^{-1} \)). Regression parameters and sensitivity are summarized in Table 2.4.1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method A</th>
<th>Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}}, \text{ nm} )</td>
<td>520</td>
<td>610</td>
</tr>
<tr>
<td>Beer’s law limits, ( \mu g \text{ mL}^{-1} )</td>
<td>0.5 – 5.0</td>
<td>2.5 – 20.0</td>
</tr>
<tr>
<td>Molar absorptivity, ( L \text{ mol}^{-1} \text{ cm}^{-1} )</td>
<td>3.4\times10^4</td>
<td>7.4\times10^3</td>
</tr>
<tr>
<td>Sandell sensitivity, ( \mu g \text{ cm}^{-2} )</td>
<td>0.0078</td>
<td>0.036</td>
</tr>
<tr>
<td>Limit of detection, ( \mu g \text{ mL}^{-1} )</td>
<td>0.04</td>
<td>0.34</td>
</tr>
<tr>
<td>Limit of quantification, ( \mu g \text{ mL}^{-1} )</td>
<td>0.13</td>
<td>1.03</td>
</tr>
<tr>
<td>Regression equation, ( Y^* )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>-0.007</td>
<td>-0.010</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.1310</td>
<td>0.029</td>
</tr>
<tr>
<td>Correlation coefficient, (r)</td>
<td>0.9997</td>
<td>0.9998</td>
</tr>
<tr>
<td>( S_a )</td>
<td>0.006</td>
<td>0.004</td>
</tr>
<tr>
<td>( S_b )</td>
<td>0.002</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

\( Y^* = a+bX \), where \( Y \) is the absorbance and \( X \) concentration in \( \mu g \text{ mL}^{-1} \).

\( S_a \) = Standard deviation of intercept.

\( S_b \) = Standard deviation of slope.
Intra-day and inter-day accuracy and precision

The precision of the methods was evaluated in terms of intermediate precision (intra-day and inter-day). Three different concentrations of ZDV (within the Beer’s law range) were analysed in seven replicates during the same day (intra-day precision) and five consecutive days (inter-day precision) by preparing all solutions afresh each day. The RSD values of intra-day and inter-day precision studies for both methods showed that the precision was satisfactory (Table 2.4.2).

The accuracy was evaluated as percentage relative error between the measured mean concentrations and added concentrations of ZDV (Bias, %). The results obtained for three different concentrations are shown in Table 2.4.2, from which it is clear that the accuracy is fairly good in both the methods.

Table 2.4.2. Intra-day and inter-day accuracy and precision studies

<table>
<thead>
<tr>
<th>Method*</th>
<th>ZDV taken</th>
<th>Intra-day accuracy and precision(^\text{a})</th>
<th>Inter-day accuracy and precision(^\text{b})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ZDV</td>
<td>RE, %</td>
<td>RSD, %</td>
</tr>
<tr>
<td>Titrimetry</td>
<td>3.0</td>
<td>3.05</td>
<td>1.67</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>5.03</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>9.01</td>
<td>0.11</td>
</tr>
<tr>
<td>Spectrophotometric method A</td>
<td>1.5</td>
<td>1.49</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>2.55</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>3.47</td>
<td>0.86</td>
</tr>
<tr>
<td>Spectrophotometric method B</td>
<td>7.5</td>
<td>7.55</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>12.40</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>17.5</td>
<td>17.61</td>
<td>0.63</td>
</tr>
</tbody>
</table>

RE, relative error; RSD, relative standard deviation.
\(^*\)In titrimetry, ZDV taken/found are in mg, while in the spectrophotometric methods, the quantities are in \(\mu\)g \(\text{mL}^{-1}\).
\(\text{a. } n=7; \text{ b. } n=5.\)

Robustness and ruggedness

In titrimetry, the reaction time was slightly altered 8, 10, and 12 min and the acid volumes were also changed (4, 5 and 6 mL). These small variations had no effect on the stoichiometry as shown by the intermediate precision expressed as percent RSD which was less than 3%. In spectrophotometry, acid volumes (1±0.1mL) and reaction time (15±1 min) were used to study the robustness. These marginal variations had no effect on the results as shown by the % RSD values of < 2.5(acid) and ≤ 1.5
(reaction time) demonstrating the robustness of the developed methods. The ruggedness of the methods was assessed by calculating the RSD for results obtained by performing the analysis using three different instruments and by three different persons. The inter-instrumental RSD values ranged from 2.5-3.5% whereas the interpersonal RSD values varied from 2.5-4.9% for three concentrations of ZDV employed for accuracy and precision. The study suggested that the methods were robust as well as rugged.

Selectivity

To evaluate the usefulness of the methods, the effect of excipient and additives, which often accompany ZDV in tablet/capsule, was studied. A synthetic mixture with the composition: ZDV (100 mg); NaCl (200 mg); talc (50 mg); starch (150 mg); lactose (200 mg); titanium dioxide (50 mg); sodium alginate (50 mg) and magnesium stearate (25 mg), was prepared and the active component (ZDV) was extracted with water as described under procedure for tablets and analysed as recommended procedure. The percentage recoveries (n=5) of ZDV was found to be 98.8±3.1 for titrimetry, 97.5±1.8 for spectrophotometric method A and 98.1±2.1 for spectrophotometric method B, indicating high selectivity for determining the studied drug in formulations.

Application to analysis of commercial samples

In order to check the validity of the proposed methods, ZDV was determined in some commercial formulations. Table 2.4.3 gives the results of the determination from which it is clear that there is close agreement between the results obtained by the proposed methods and the label claim. The results were also compared statistically by a Student’s t-test for accuracy and variance ratio F-test for precision with those of the reference method [5] at 95% confidence level. The calculated t- and F-values (Table 2.4.3.) did not exceed the tabulated values (t=2.77, F=6.39) for four degrees of freedom indicating that there was no significant difference between the proposed methods and the reference method in respect to accuracy and precision.
Table 2.4.3. Results of determination of zidovudine in formulations and statistical comparison with the reference method

<table>
<thead>
<tr>
<th>Formulation Brand name</th>
<th>Nominal amount, mg</th>
<th>% Found* ± SD</th>
<th>Reference method</th>
<th>Titrimetry</th>
<th>Method A</th>
<th>Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIRO-Z (Tablets)</td>
<td>100</td>
<td>99.58±0.35</td>
<td>98.8±0.82</td>
<td>98.90±0.69</td>
<td>100.6±0.85</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>t=2.11</td>
<td>t=2.07</td>
<td>t=2.69</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F=5.49</td>
<td>F=3.89</td>
<td>F=5.90</td>
<td></td>
</tr>
<tr>
<td>ZIDO-H (Capsules)</td>
<td>300</td>
<td>99.91±0.56</td>
<td>100.3±0.45</td>
<td>98.62±1.01</td>
<td>100.1±1.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>t=1.21</td>
<td>t=2.60</td>
<td>t=0.38</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F=1.55</td>
<td>F=3.25</td>
<td>F=3.32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>102.3±0.51</td>
<td>101.6±0.39</td>
<td>101.95±1.02</td>
<td>100.6±1.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>t=2.46</td>
<td>t=0.72</td>
<td>t=0.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F=1.71</td>
<td>F=4.00</td>
<td>F=5.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>100.5±0.62</td>
<td>101.1±0.55</td>
<td>99.95±1.32</td>
<td>101.9±1.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>t=1.62</td>
<td>t=0.90</td>
<td>t=2.27</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F=1.27</td>
<td>F=4.53</td>
<td>F=4.60</td>
<td></td>
</tr>
</tbody>
</table>

*Mean value of five determinations
#Marketed by: a. Ranbaxy Ltd. India.; b. Genix Ltd.
Tabulated t-value at 95% confidence level is 2.77
Tabulated F-value at 95% confidence level is 6.39.

Recovery Study

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies (Table 2.4.4.). Pre-analysed tablet or capsule powder was spiked with pure ZDV at three different levels and the total was found by the proposed methods. Each determination was repeated three times. The recovery of the pure drug added was quantitative (98.3-105.1%).

Table 2.2.4- Results of recovery experiments by standard addition method

<table>
<thead>
<tr>
<th>Formulation Studied</th>
<th>Titrimetry</th>
<th>Spectrophotometric method A</th>
<th>Spectrophotometric method B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ZDV in tablet, µg mL⁻¹</td>
<td>Pure ZDV added, µg mL⁻¹</td>
<td>Total found, µg mL⁻¹</td>
</tr>
<tr>
<td>VIRO-Z 100</td>
<td>3.922</td>
<td>1.0</td>
<td>4.93</td>
</tr>
<tr>
<td></td>
<td>3.922</td>
<td>3.0</td>
<td>6.97</td>
</tr>
<tr>
<td></td>
<td>3.922</td>
<td>5.0</td>
<td>9.04</td>
</tr>
<tr>
<td>ZIDO-H 300</td>
<td>4.084</td>
<td>1.0</td>
<td>5.11</td>
</tr>
<tr>
<td></td>
<td>4.084</td>
<td>3.0</td>
<td>7.03</td>
</tr>
<tr>
<td></td>
<td>4.084</td>
<td>5.0</td>
<td>9.34</td>
</tr>
</tbody>
</table>

*Mean value of three determinations.
SECTION 2.5

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC
DETERMINATION OF ZIDOVUDINE IN PHARMACEUTICALS*

2.5.1.0. INTRODUCTION

High performance liquid chromatography (HPLC) is probably the single most analytical technique used today. This is due mainly to the extensive versatility of the technique [236]. Analysis of pharmaceutical preparations by chromatography can be traced back to 1922 [237]. By 1955, descending and ascending paper chromatography had been described in “The United States Pharmacopeia” (USP) for the tentative identification of drug products [238].

Historically, HPLC can be traced back to the amino acid analyzers of the early 1960s. By 1975, liquid chromatographic instrumentation was described in the US. Pharmacopoeia. Since that time, HPLC has become the most popular chromatographic technique in the pharmaceutical Industry laboratory [239].

Although modern HPLC became popular in 1969, it was not widely accepted by pharmaceutical analysts until several years later. However, the usefulness of HPLC technique for pharmaceutical analysis was not appreciated by many practitioners in the pharmaceutical industry until the first HPLC systems capable of quantitative analysis became commercially available.

HPLC techniques, on the other hand, offer enhanced detection sensitivity, improved accuracy, and reproducibility of drug analysis in the course of drug research, development and quality control testing of marketed drug products. Many wet chemistry and classical test methods for existing drug products have also been replaced by HPLC methods for more accurate measurements, better precision and much faster analytical run time. This translates into lower cost per test in Research and Development and Quality Control Laboratories [240].

The literature survey on the HPLC methods for the assay of zidovudine is presented in Section 2.0.2 from which it is obvious that the currently available HPLC method [3] is less sensitive.

*This work has been published in Proc. Indian Natl. Sci. Acad., 2007, 73(2), 51-54
By introducing certain modifications in respect of column and mobile phase composition, the author has been able to develop an HPLC method for zidovudine which does not require an internal standard. The method is applicable over a wide dynamic concentration range. The details of method development and method validation are presented in this Section.

2.5.2. EXPERIMENTAL

2.5.2.1. Apparatus

The chromatographic system consisted of an Agilent 1100 series chromatograph equipped with an in-built solvent degasser, quaternary pump, photo diode array detector with variable injector and auto sampler, and a reversed phase 5 µm Hypersil ODS column (250x4.6 mm, i.d.). Data was recorded and evaluated by use of Class-VP 5.032 software.

2.5.2.2. Reagents and Standards

All chemicals used were of analytical reagent grade and HPLC grade acetonitrile (Merck. Ltd, Mumbai) was used. Distilled water filtered through 0.45 µm filter (Millipore) was used to prepare solutions.

Mobile phase: Prepared by mixing water and acetonitrile in the ratio, 85:15 (v/v). The mobile phase was used as a diluent for the sample preparations.

Standard drug solution

For the study, an accurately weighed 50 mg of ZDV was dissolved in and diluted to volume with the diluent solution in a 50 mL calibrated flask to obtain a concentration of 1000 µg mL⁻¹ ZDV.

2.5.3. PROCEDURES

Chromatographic conditions

The separation was achieved at ambient temperature on the column using the mobile phase at a flow rate of 1 mL min⁻¹. The detector wavelength was set at 265 nm with a sensitivity of 0.2 a.u.f.s.

Calibration graph

Working standard solutions equivalent to 50 to 750 µg mL⁻¹ ZDV were prepared by appropriate dilution of stock standard solution (1000 µg mL⁻¹) with the
diluent solution. Twenty µl aliquot of each solution was injected automatically on to the column in duplicate and the chromatograms were recorded. Calibration graph was prepared by plotting the mean peak area versus concentration of ZDV.

The concentration of the unknown was read from the calibration graph or computed from the regression equation derived from the mean peak area-concentration data.

**Assay in dosage forms**

A quantity of capsule/tablet powder equivalent 100 mg of ZDV was accurately weighed into a 100 mL calibrated flask, 60 mL of diluent solution added and content shaken for 20 min; then, the volume was diluted to the mark and mixed well. A small portion of the extract (say 10 mL) was withdrawn and filtered through 0.45 µm filter to ensure the absence of particulate matter. The filtered solution was appropriately diluted with the diluent solution for analysis as described already.

**2.5.4. RESULTS AND DISCUSSION**

**2.5.4.1. Method development**

A solution of ZDV was injected in duplicate on to the column and was monitored by UV-detection at 265 nm. A mobile phase consisting of water-acetonitrile (85:15) was used after several preliminary experiments. At a flow rate of 1.0 mL min\(^{-1}\), the retention time was 9.1 min (Fig 2.5.1). Under the described experimental conditions, the peak was well-defined and free from tailing. ZDV was determined by measuring the peak area.

![Fig.2.5.1. Typical chromatogram](image-url)
2.5.4.2. Method Validation

Specificity

Method specificity was checked by comparing the chromatograms obtained for pure ZDV solution, synthetic mixture, tablet solution and placebo blank. It was found that there was no interference with the analyte peak and system suitability parameters such as theoretical plates and tailing factor were found to be 7047 and 1.22, respectively.

Linearity range

Linearity was assessed in the range of 50 to 150% of the working level concentration including working level concentration. First and last level of linearity was carried out in six replicates and other levels in duplicates. The linearity coefficient of mean response of replicate determination plotted against respective concentration. The percent y-intercept as obtained from the linearity data was less than 2%. The % RSD for peak area response of six replicates of first and last level was less than 2.0% and 1.0% for retention time.

A plot of mean peak area against concentration (Fig 2.5.2) gave a linear relationship \( r=0.9999, n=8 \) over the concentration range 50-750 \( \mu \text{g mL}^{-1} \). Using the regression analysis, the linear equation, \( Y=13.9065 + 20.0012 X \) was obtained, where, \( Y \) is the mean peak area and \( X \) concentration in \( \mu \text{g mL}^{-1} \).

Fig.2.5.2. Linearity curve
Accuracy and Precision

To determine the intra-day accuracy and precision, pure ZDV solution at three different concentrations was analysed in seven replicates on the same day. The percent relative error which is an index of accuracy is <1.0% and is indicative of good accuracy. The peak area based RSD was less than 1% and retention time based RSD was less than 0.2% indicating high accuracy and precision of the method. These results are presented in Table. 2.5.1. The inter-day precision was established by performing analyses over a period of five days on solutions prepared afresh each day. The peak-area based and retention-time-based RSD values were <2.0 % and <1 %, respectively.

**Table 2.5.1- Accuracy and intra-day precision**

<table>
<thead>
<tr>
<th>ZDV Taken µg mL⁻¹</th>
<th>ZDV found* µg mL⁻¹</th>
<th>RE %</th>
<th>RSD $ %</th>
<th>RSD @ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>198.2</td>
<td>0.90</td>
<td>0.63</td>
<td>0.10</td>
</tr>
<tr>
<td>400</td>
<td>395.8</td>
<td>1.05</td>
<td>0.22</td>
<td>0.11</td>
</tr>
<tr>
<td>600</td>
<td>595.5</td>
<td>0.75</td>
<td>0.16</td>
<td>0.11</td>
</tr>
</tbody>
</table>

RE, relative error; RSD, Relative standard deviation; *Mean value of seven determinations; $ Based on peak area; @ Based on retention time

Ruggedness (Intermediate Precision)

Intermediate precision of six replicate determination of assay of a sample was analysed by different analyst with different instrument in different day after specifying the system suitability of the method. The %RSD of assay was less than 2.0% and the cumulative %RSD of assay of precision study and intermediate precision was also less than 2.0%.

Robustness

Robustness was done by altering deliberately two critical parameters by minor variation.

1. Flow rate was changed to 1.01 mL min⁻¹.
2. The wavelength was changed to 266 nm.

The %RSD for peak area response was less than 2.0% and 1.0% for retention time. The cumulative %RSD of assay of precision study and Robustness was also less than 2.0%.
**Limit of Quantitation and Limit of Detection**

LOQ and LOD were established based on signal-to-noise ratio, performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples. The signal to noise ratio of 3:1 and 10:1 were taken as LOD and LOQ, respectively and were found to be 6.0 µg mL⁻¹ and 20.0 µg mL⁻¹, respectively.

**Application**

The method was applied to the determination of ZDV in dosage forms. The results obtained are presented in Table 2.5.2 and compare well with label claim. The results were also compared statistically with those obtained by a reference method [5] by applying Student’s t-test for accuracy and F-test for precision. At the 95% confidence level, the calculated t- and F-values did not exceed the tabulated values (t = 2.77 and F = 6.39) suggesting that the proposed methods are as accurate and precise as the reference method.

**Table 2.5.2- Results of determination of zidovudine in formulations and statistical comparison with the reference method**

<table>
<thead>
<tr>
<th>Formulation Brand name*</th>
<th>Nominal amount, mg</th>
<th>% found* ± SD</th>
<th>Reference method</th>
<th>Proposed Method</th>
<th>t-value</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIRO-Z (TABLETS)</td>
<td>100</td>
<td>102.3±0.62</td>
<td>101.2±1.39</td>
<td>1.73</td>
<td>5.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>97.2±1.06</td>
<td>98.3±1.48</td>
<td>1.37</td>
<td>1.95</td>
<td></td>
</tr>
<tr>
<td>ZIDO-H (CAPSULES)</td>
<td>100</td>
<td>101.3±0.62</td>
<td>99.8±1.32</td>
<td>2.44</td>
<td>4.53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>100.6±0.85</td>
<td>99.8±1.61</td>
<td>1.03</td>
<td>3.59</td>
<td></td>
</tr>
</tbody>
</table>

*Mean value of seven determinations #Marketed by: a. Ranbaxy Ltd. India.; b. Genix Ltd.
Tabulated t-value at 95% confidence level is 2.77
Tabulated F-value at 95% confidence level is 6.39.

**Recovery study**

The accuracy and validity of the proposed methods were further ascertained by performing recovery experiments. Pre-analysed capsule/tablet powder was spiked with pure ZDV at three different levels and the total was found by the proposed methods. Each determination was repeated three times. The recovery of pure drug added was quantitative (Table 2.5.3) and revealed that co-formulated substances did not interfere in the determination.
<table>
<thead>
<tr>
<th>Formulation Studied</th>
<th>ZDV in formulation, µg mL(^{-1})</th>
<th>ZDV added, µg mL(^{-1})</th>
<th>Total found µg mL(^{-1})</th>
<th>Pure ZDV recovered* %</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIRO-Z 100 (Tablets)</td>
<td>303.6</td>
<td>100</td>
<td>402.8</td>
<td>99.2</td>
</tr>
<tr>
<td></td>
<td>303.6</td>
<td>200</td>
<td>510.0</td>
<td>103.2</td>
</tr>
<tr>
<td></td>
<td>303.6</td>
<td>300</td>
<td>604.5</td>
<td>100.3</td>
</tr>
<tr>
<td>ZIDO-H 300 (Capsules)</td>
<td>299.4</td>
<td>100</td>
<td>397.9</td>
<td>98.5</td>
</tr>
<tr>
<td></td>
<td>299.4</td>
<td>200</td>
<td>494.8</td>
<td>97.7</td>
</tr>
<tr>
<td></td>
<td>299.4</td>
<td>300</td>
<td>603.0</td>
<td>101.2</td>
</tr>
</tbody>
</table>

*Mean value of three determinations
SECTION 2.6

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC –MASS
SPECTROMETRIC DETERMINATION OF ZIDOVUDINE IN URINE*

2.6.1.0. INTRODUCTION

High performance liquid chromatography-mass spectrometry (LC-MS) is a powerful technique used for many applications and has very high sensitivity and specificity. Generally, its application is oriented towards the specific detection and potential identification of chemicals in the presence of other chemicals (in a complex mixture).

There are a lot of mass analysers that can be used in LC/MS. Single quadropole, Triple Quadrupole, Ion trap, TOF(Time of flight) and Quadrupole-time of flight(Q-TOF). The interface is most often an electrospray ion source or variant such as nanospray source; however, fast atom bombardment, thermospray and atmospheric pressure chemical ionisation interfaces are also used [241].

LC-MS is very commonly used in pharmacokinetic studies in pharmaceuticals. These studies tell us how quickly a drug will be cleared from the Hepatic Blood flow, and organs of the body. MS is used for this due to high sensitivity and exceptional specificity compared to UV, and quick analysis time. The major advantage MS has been the use of Tandem MS-MS. It is common now to have analysis times of 1 minute or less by MS-MS detection, compared to over 10 minutes with UV detection [242,243].

LC-MS is frequently used in the drug development at many different stages including peptide mapping, glycoprotein mapping, natural products dereplication, bioaffinity screening, *in vivo* drug screening, metabolic stability screening, metabolite identification, impurity identification, degradant identification, quantitative bio analysis, and quality control [244].

The literature survey revealed that no method using LC-MS technique has been reported for the determination of ZDV in human urine. The author has developed an HPLC-MS-MS method for the determination of ZDV in human urine. The details of method development and method validation are presented in this Section, 2.6.

*This work has been communicated to *J. Mex. Chem. Soc.*,.
2.6.2. EXPERIMENTAL

2.6.2.1. Apparatus

The LC-MS-MS analysis was performed with an API 2000 (Applied Biosystems) coupled to an HPLC system comprising an Agilent 1100 series low pressure quaternary gradient pump with degasser, autosampler, and the column oven. Data was processed through analyst software.

2.6.2.2. Reagents and Standards

All chemicals used were of analytical reagent grade and HPLC grade acetonitrile and methanol (Merck, Ltd, Mumbai) were used. Distilled water filtered through 0.45 µm filter (Millipore, Bangalore) was used to prepare the solutions. For sample preparations Oasis HLB cartridge (Waters, Bangalore) was used.

**Mobile phase**: Prepared by mixing water and acetonitrile in the ratio of 20:80 (v/v). Methanol was used as the diluent.

A stock standard containing 100 µg mL\(^{-1}\) ZDV solution was prepared by dissolving accurately weighed 10 mg of pure drug in diluent and diluting to 100 mL in a calibrated flask with diluent. It was subsequently diluted to obtain a working concentration.

2.6.3. PROCEDURES

**LC-MS conditions**:

The chromatographic separation was achieved at ambient temperature (25° C) on the column (Symmetry C\(_{18}\), 3.5µm; 75x4.6 mm i.d) using the mobile phase of water and acetonitrile in the ratio of 20:80(v/v) at a flow rate of 0.4 mL min\(^{-1}\). The mobile phase was degassed before use.

Quantitation was performed using multiple reaction monitoring (MRM) of the transitions of 268 m/z → 127.1 m/z for zidovudine and 225.2 m/z → 127.1 m/z for stavudine (Internal Standard, Fig. 2.6.1) with a scan time of 0.2 s per transition. Fig. 2.6.2 shows the product ion spectra of [M+H]\(^+\) for zidovudine and stavudine.
Fig. 2.6.1. Structure of stavudine

Fig. 2.6.2. Full scan product ion spectra of zidovudine (A) and stavudine (B)
In order to optimize all the MS parameters, a standard solution (1 µg mL\(^{-1}\)) of the analyte and IS (1 µg mL\(^{-1}\)) was infused into the mass spectrometer.

Analysis was performed in positive mode (ESI) with a turbo ion spray interface under the conditions: ion source potential, 5500 V; declustering potential, 70 V; focusing potential, 400 V, capillary temperature, 350°C; entrance potential, 10 V with nitrogen as nebuliser gas at 25 Psi. The column eluent was introduced into the electron spray ionization chamber of the mass spectrometer with a split ratio of 3:7. Mass fragmentation studies were performed by maintaining the normalized collision energy at 15 eV.

Sample preparation

The urine samples collected from healthy volunteers were spiked with known concentration of ZDV and I.S. and the resulting sample was diluted 1:1 with water prior to loading on solid phase extraction(SPE) cartridge (Oasis HLB-60 mg). The SPE cartridge was conditioned with 1 mL of methanol and equilibrated with 1mL of water, and then 1 mL of diluted urine was loaded on. The cartridge was then washed with 1mL of 10% methanol and then eluted with 100% methanol. Sample was evaporated with nitrogen at 40° C. and reconstituted the residue with water: methanol (1:1) to 500 µL. Then 20 µL of the above solution was injected into LC-MS-MS for analysis. This procedure was carried out under yellow monochromatic light.

Preparation of standard and quality control samples

Stock solutions of ZDV and IS (both 10 µg mL\(^{-1}\)) were prepared in methanol and diluted to 1000 ng mL\(^{-1}\) with methanol:water(1:1). The stock solutions were kept in amber colored bottle and stored in refrigerator when not in use.

Calibration graph:

Calibration curve was prepared by diluting the stock solution with methanol:water (1:1) to get appropriate concentration. From this dilution, 20 µL of appropriate standard solution was added to 1 mL of urine blank to get an effective concentration of 10, 20, 80, 100, 300, 500,850 and 1000 ng mL\(^{-1}\) ZDV.
The quality control (QC) samples were separately prepared in the blank urine sample at the concentrations of 150, 350 and 750 ng mL\(^{-1}\), respectively. The spiked urine samples (standards and quality controls) were then treated for SPE.

2.6.4. RESULTS AND DISCUSSIONS

Mass spectrometry

The signal intensity obtained for ZDV in positive mode was much higher than that in negative mode. Then, the possibility of using electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) sources under positive ion detection mode was evaluated during the early stage of method development. ESI spectra revealed higher signals for the molecule compared to APCI source. Further assay development was therefore limited to ESI source. The Q1 full spectra of ZDV and IS were dominated by protonated molecules [M+H]\(^+\) and no significant solvent adduct ions and fragments ions were observed. The tuning of the ESI source such as capillary temperature, flow of sheath and auxiliary gas (N\(_2\)) and spray voltage on the transition of ZDV and IS further improved the sensitivity.

Chromatography

Although in the aspect of chromatographic separation the determination of the analyte was not interfered by endogenous substances in the urine sample, yet the ionization of the analyte, especially of low concentration, was easily suppressed, which resulted in the linearity in narrow concentration range. In order to avoid the ion suppression induced by endogenous substances, the influence of the mobile phase that composed of different percentage of organic phase on the ion suppression was evaluated during the experiment. It was found that when mobile phase consisted of water-acetonitrile (20:80, v/v), the spiked sample demonstrated good linearity between 10 to 1000 ng mL\(^{-1}\) for ZDV. Under the present chromatographic conditions, the run time of each sample was 4.0 min. The retention times were 1.98 min and 1.97 min for ZDV and IS, respectively (Fig. 2.6.3.).
Fig. 2.6.3. Representative chromatograms of zidovudine (10 ng mL$^{-1}$) and IS (500 ng mL$^{-1}$)

2.6.4.1. Method Validation

Specificity

There was no significant interferences or ion suppression from endogenous substances observed at the retention times of the analytes in the blank chromatograms of ZDV and IS (Fig. 2.6.4).
Fig. 2.6.4. Representative blank chromatograms for zidovudine (A) and IS (B)

**Linearity of calibration curve and lower limit of quantification**

Urine samples were quantified using ratio of the peak area of ZDV to that of IS as the assay parameter. Peak area ratios were plotted against ZDV concentrations and standard curve in the form of \( Y = a + bX \) was calculated using weighed \((1/x^2)\) least squares linear regression.

The linear range of calibration curve was 10-1000 ng mL\(^{-1}\) for the analyte. The representative calibration curve was as follows: \( Y=0.00665 +0.0651x \) \((r= 0.9987)\).

The lower limit of quantification was defined as the lowest concentration on the calibration curve for which the acceptable accuracy of ±15% and a precision below ±15% were obtained. The present LC-MS-MS method offered an LLOQ of 10 ng mL\(^{-1}\).

**Intra-day and inter-day accuracy and precision**

The intra-day accuracy and precision were assessed by determining QC samples in a set of six replicates within one day. The accuracy was expressed by \((\text{mean observed concentration})/(\text{spiked concentration})\times100\%\) and the precision by relative standard deviation (RSD). The inter-day precision was established by performing analyses over a period of five days on solutions prepared afresh each day.

Table-2.6.1 summarizes the precision and accuracy for the ZDV evaluated by assaying the QC samples.
Table 2.6.1- Accuracy and intra-day precision

<table>
<thead>
<tr>
<th>ZDV taken ng mL(^{-1})</th>
<th>ZDV found* ng mL(^{-1})</th>
<th>Intra-day RSD, %</th>
<th>Inter-day RSD, %</th>
<th>RE %</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>148.9</td>
<td>7.9</td>
<td>9.5</td>
<td>0.73</td>
</tr>
<tr>
<td>350</td>
<td>348.6</td>
<td>6.6</td>
<td>7.6</td>
<td>0.40</td>
</tr>
<tr>
<td>750</td>
<td>748.2</td>
<td>7.6</td>
<td>6.9</td>
<td>0.24</td>
</tr>
</tbody>
</table>

RE, relative error; RSD, Relative standard deviation; *Mean value of seven determinations

Recovery study

Absolute recoveries of ZDV at three QC levels were determined by assaying the samples as described above and comparing the peak areas of both ZDV and IS with those obtained from direct injection of the compounds dissolved in the blank urine. The recovery of ZDV, determined at three concentrations (150, 350, 750 ng mL\(^{-1}\)) were 85.4±5.4%, 89.4±6.4% and 86.4±4.4% (n=6) respectively.
Table 2.7.1. Comparison of Performance Characteristics of Proposed Methods with the Existing Methods

### A. Titrimetry

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Titrant</th>
<th>Titration conditions</th>
<th>Range, mg</th>
<th>Remarks</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No titrimetric method has been reported for ZDV.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>KBrO₃-KBr mixture</td>
<td>Iodometric back titration method in HCl medium</td>
<td>2.0-10.0</td>
<td>Uses an oxidant which is highly stable solution</td>
<td>This work</td>
</tr>
<tr>
<td>2.</td>
<td>NBS</td>
<td>Iodometric back titration method in HCl medium</td>
<td>4.0-15.0</td>
<td>Wide dynamic linear range; but NBS requires daily standardisation</td>
<td>This work</td>
</tr>
<tr>
<td>3.</td>
<td>CAT</td>
<td>Iodometric back titration method in HCl medium</td>
<td>3.0-10.0</td>
<td>Applicable over a micro scale.</td>
<td>This work</td>
</tr>
</tbody>
</table>

### B. Spectrophotometry

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Reagent*</th>
<th>λₘₐₓ, nm</th>
<th>Linear range, µg mL⁻¹</th>
<th>ε, l mol⁻¹ cm⁻¹</th>
<th>Remarks</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No visible spectrophotometric method has been reported for ZDV.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>a) KBrO₃-KBr- methyl orange</td>
<td>520</td>
<td>0.5-5.0</td>
<td>4 × 10⁴</td>
<td>Involves no extraction/ heating step; Use highly stable solutions; measured species highly stable very sensitive.</td>
<td>This work</td>
</tr>
<tr>
<td></td>
<td>b) KBrO₃-KBr- indigocarmine</td>
<td>610</td>
<td>2.0-20</td>
<td>8.6 × 10³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>a) NBS- methyl orange</td>
<td>520</td>
<td>0.5-6.0</td>
<td>2.7 × 10⁴</td>
<td>Sensitive; but NBS unstable in solution</td>
<td>This work</td>
</tr>
<tr>
<td></td>
<td>b) NBS- indigocarmine</td>
<td>610</td>
<td>2.5-20</td>
<td>8.2 × 10³</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### C. High Performance Liquid Chromatography.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Chromatographic conditions</th>
<th>$\lambda_{\text{max}}$, nm</th>
<th>Linear range, $\mu\text{g mL}^{-1}$</th>
<th>Remarks</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>C18 column with mobile phase water: MeOH(77:23) at 1.4 mL min$^{-1}$</td>
<td>265</td>
<td>25-500</td>
<td>Sensitive; narrow linear range</td>
<td>3</td>
</tr>
<tr>
<td>2.</td>
<td>C18 column</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>3.</td>
<td>Hypersil ODS (250 × 4.6 mm, i.d.) column with mobile phase H$_2$O:CH$_3$CN (85:15) at 1.0 mL min$^{-1}$.</td>
<td>265</td>
<td>50-750</td>
<td>Uses no internal standard, wide linear dynamic range of applicability</td>
<td>This work</td>
</tr>
</tbody>
</table>

### D. Liquid chromatography-Mass spectrometry

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Chromatographic conditions</th>
<th>Linear range, ng mL$^{-1}$</th>
<th>Remarks</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Symmetry C$_{18}$, (3.5µm; 75x4.6 mm i.d) with mobile phase of water and acetonitrile( 20:80) at 0.4 mL/min</td>
<td>10-1000</td>
<td>Wide linear dynamic range of applicability, high sensitivity</td>
<td>This work</td>
</tr>
</tbody>
</table>

* NBS.N-bromosuccinimide; CAT. Chloramine-T; SAA. Sulphanilic acid
A comparison of performance characteristics of the proposed titrimetric, spectrophotometric and HPLC methods with those of the existing methods is presented in Table 2.7.1 above. To the best of the author’s knowledge, no titrimetric method and visible spectrophotometric method have ever been reported for zidovudine, and the proposed methods employing bromate-bromide, NBS, and CAT as reagents are simple, use mild experimental conditions and are applicable over a micro scale.

The outstanding performance characteristics of the proposed methods are simplicity, sensitivity and wide dynamic linear concentration range of applicability. Except the methods using bromate-bromide/NBS/CAT-indigocarmine and NBS-metol-SAA as reagents, the other methods have better sensitivity ($\epsilon$ values in the range $2.7 \times 10^4$ - $4.0 \times 10^4$ Lmol$^{-1}$cm$^{-1}$). The sensitivity of the proposed spectrophotometric methods in terms of linear range of applicability is found to be better than the existing as well as the present HPLC method. A significant advantage of the proposed methods is that the absorbance is measured at longer wavelength (520-610 nm) where the interference from the associated inactive ingredients is usually far less than at shorter wavelengths. Additionally, all the methods are based on the ultimate measurement of the coloured species which is highly stable in solution.

With the relative error (RE) and relative standard deviation (RSD) values of under 2.5%, all the titrimetric and spectrophotometric methods are fairly accurate and precise. While comparing the results of the proposed methods with those the reference method, discrepancies are observed in a couple of instances, particularly with respect to the Student’s t-value, and such deviations can be largely ascribed to indeterminate errors. The proposed titrimetric and spectrophotometric methods rely on the use of inexpensive and eco-friendly chemicals, and simple instrumentation.

Of the oxidimetric reagents employed in the study, only NBS needs daily standardization, and the remaining two, $\text{BrO}_3^-$, $\text{Br}^-$ and CAT are highly stable in solution. The proposed methods using NBS and $\text{BrO}_3^-$, $\text{Br}^-$ are not specific and are not ideally suited for assay in combined dosage forms, where any substance with active hydrogen and unsaturation would cause interference. Fortunately, the dosage forms used in the present study were devoid of such species as shown by the results of assay as well as of recovery experiments.
A single most significant advantage of the proposed HPLC method compared to the titrimetric and spectrophotometric methods is its remarkable specificity in addition to speed, accuracy and precision. Thus, while the titrimetric and spectrophotometric methods, because of their non-specificity are applicable to single dosage forms, the HPLC method, under the described chromatographic conditions, is applicable to single as well as combined dosage forms. However, the method possess wide linear dynamic range in comparison with the reported HPLC method [3].

Nowadays LC-MS is the single most widely used technique for bioanalytical studies which can be used in complex mixtures. The proposed method is most sensitive with a LLOQ of 10 ng mL⁻¹.

Thus, three titrimetric, seven spectrophotometric and one HPLC methods for the assay of zidovudine in pharmaceuticals and one LC-MS method for determination of zidovudine in human urine have been developed and validated according to the current ICH guidelines. The methods have been demonstrated to be fairly accurate and precise in addition to being highly sensitive (except HPLC and a couple of spectrophotometric methods). The titrimetric and spectrophotometric methods can usefully be employed in routine use in areas /countries which lack modern instrumental facilities such as HPLC, LC-mass spectrometry, spectrofluorimetry, capillary electrophoresis, etc.,
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