Titrimetric and spectrophotometric determination of zidovudine in pharmaceuticals using chloramine-T and two dyes

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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 seemed 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 indigocarmine and measuring the absorbance at 610 nm (Method B). In all the methods, the amount of CAT reacted corresponds to the amount of ZDV. In titrimetric method, the reaction follows a reaction stoichiometry of 1:1 (ZDV: CAT), and is applicable over a range 3-10 mg of ZDV. In spectrophotometric methods, the absorbance is found to increase linearly with concentration of ZDV. The systems obey Beer’s law for 0.5-5.0 and 2.5-20.0 μg mL⁻¹ for method A and method B, respectively. The apparent molar absorptivities are calculated to be 3.4×10⁴ and 7.4×10³ L mol⁻¹ cm⁻¹ for method A and method B, respectively. The methods were successfully applied to the assay of ZDV in tablet and capsule formulations.

Keywords: Zidovudine, Assay, Titrimetry, Spectrophotometry, Chloramine-T, Formulations

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Zidovudine(ZDV) is chemically known as 3¹-Azido-3¹-deoxy thymidine. It is the first drug approved for the treatment of AIDS and HIV infection. ZDV is a thymidine analogue. It is phosphorylated in the body to zidovudine triphosphate, which is the active form that inhibits HIV replication. ZDV inhibits the key enzyme reverse transcriptase.

High performance liquid chromatography (HPLC) is the single most widely used technique for the determination of ZDV, but most of the studies are devoted to the determination of drug in body fluids such as human serum, human plasma, rat plasma, human plasma and urine, and rat plasma. There is only one report on the use of HPLC for the specific determination of ZDV in pure drug and marketed tablets. UV-spectrophotometry has also found application for the simultaneous determination of ZDV and lamivudine in human serum. The technique in different modes has been applied for the assay of ZDV when present alone or in combination with lamivudine in pharmaceuticals.

The present investigation aims to develop sensitive and cost-effective methods for the determination of ZDV in pure form and in dosage forms using titrimetric and spectrophotometric techniques using chloramine-T as the oxidimetric reagent.

Experimental Procedure

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

Reagents and materials
All chemicals used were of analytical reagent grade and distilled water was used to prepare all solutions. Chloramine-T solution (5 m mol L⁻¹) was 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 titrimetric investigations. For spectrophotometric investigations, the above solution was diluted appropriately with water to get 55 and 200 μg mL⁻¹ concentrations for method A and method B, respectively. To prepare 50 μg mL⁻¹ methyl orange for method A, first, a 500 μg mL⁻¹ dye solution was prepared by dissolving 59 mg of dye (s.d.fine-chem Ltd., Mumbai, assay 85%) in water and diluting to
100 mL in a calibrated flask, and filtered using glass wool. This was diluted ten-fold with water to get the required concentration. For method B, first, a 1000 μg mL⁻¹ indigo carmine solution was prepared by dissolving 112 mg of dye (s.d.fine-chem Ltd., Mumbai, 90% assay) in water and diluting to 100 mL, and filtered. This was appropriately diluted with water to get 200 μg mL⁻¹. Hydrochloric acid (2 mol L⁻¹) was prepared by diluting 43 mL of concentrated acid (s.d.fine-chem Ltd., Mumbai, Sp gr 1.18) to 500 mL with water. Sodium thiosulphate solution (0.01 mol L⁻¹) was prepared by dissolving about 2.48 g of the chemical (SISCO Chem, Industries, Mumbai) in 1 litre of water and standardized with pure potassium dichromate iodometrically. Aqueous solutions of potassium iodide (10%) and starch indicator (1%) were prepared in the usual way. Pharmaceutical grade ZDV, certified to be 99.8% pure was procured from Cipla India Ltd, Mumbai, India, and was used as received. A 1 mg mL⁻¹ solution of ZDV was prepared by dissolving accurately weighed 250 mg of pure drug in water and diluting to 250 mL with water in a calibrated flask and used for assay by titrimetry. This stock solution (1000 μg mL⁻¹) was diluted with water to get working concentrations of 10 and 50 μg mL⁻¹ ZDV for method A and method B, respectively.

Methods

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 of 2 mol L⁻¹ hydrochloric acid followed by 10 mL of 5 mol L⁻¹ 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 mol L⁻¹ sodium thiosulphate to a starch endpoint. 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.

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 of 2 mol L⁻¹ hydrochloric acid followed by 1 mL of 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.

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 mol L⁻¹ 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

An amount of 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. Then, the volume was made up to the mark with water, mixed well, and filtered using a Whatman No 42 filter paper. First 10 mL portion of the filtrate was discarded and a suitable aliquot of the subsequent portion (1 mg mL⁻¹ ZDV) was taken for assay by titrimetric procedure. The filtrate was diluted appropriately to get 10 and 50 μg mL⁻¹ concentrations for analysis by spectrophotometric method A and method B, respectively.

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 purpose.

Optimisation of experimental conditions

Titrimetry

The reaction stoichiometry was found to be 1:1(ZDV: CAT), which was unaffected in the presence of 5 to 10 mL of 2 mol L⁻¹ HCl in a total...
volume of 25 mL and 5 mL of 2 mol L\(^{-1}\) 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 m mol L\(^{-1}\) CAT solution was found adequate for the quantitative oxidation of ZDV.

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 products in acid medium has been used. 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 mL\(^{-1}\) for methyl orange and indigo carmine, respectively. A chloramine-T concentration of 5.5 \(\mu\)g mL\(^{-1}\) was found to irreversibly destroy the red colour of 5 \(\mu\)g mL\(^{-1}\) methyl orange, whereas 20.0 \(\mu\)g mL\(^{-1}\) chloramine-T was required to bleach the blue colour due to 20 \(\mu\)g mL\(^{-1}\) indigo carmine. Hence, different amounts of ZDV were reacted with 1 mL of 55 \(\mu\)g mL\(^{-1}\) chloramine-T in method A and 1 mL of 200 \(\mu\)g mL\(^{-1}\) chloramine-T in method B, followed by determination of the residual oxidant as described under the respective procedures.

Analytical data
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 mL\(^{-1}\)). Regression parameters and sensitivity are summarized in Table 1.

Method validation
Accuracy and precision
To evaluate the accuracy and precision of the methods, pure drug solution at 5 mg, 2.5 \(\mu\)g mL\(^{-1}\) and

<table>
<thead>
<tr>
<th>Formulation brand name(^{#})</th>
<th>Nominal amount, mg</th>
<th>100</th>
<th>99.58±0.35</th>
<th>98.04±0.82</th>
<th>98.90±0.69</th>
<th>100.6±0.85</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Reference method</td>
<td>Titrimetry</td>
<td>Method A</td>
<td>Method B</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(t=4.16)</td>
<td>(t=2.07)</td>
<td>(t=2.69)</td>
<td>(t=5.90)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(F=5.49)</td>
<td>(F=3.89)</td>
<td>(F=4.90)</td>
<td>(F=5.90)</td>
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VIRO-Z\(^{\#}\)
(Tables)

|                             |                   | 300 | 99.91±0.56 | 100.3±0.45 | 98.62±1.01 | 102.1±1.02 |
|                             |                   |     | Reference method | Titrimetry | Method A | Method B |
|                             |                   |     | \(t=1.21\) | \(t=2.60\) | \(t=4.38\) | \(t=3.32\) |
|                             |                   |     | \(F=1.55\) | \(F=3.25\) | \(F=3.25\) | \(F=3.32\) |

100 | 102.3±0.51 | 101.6±0.39 | 101.95±1.02 | 100.6±1.16 |
|     | \(t=2.46\) | \(t=0.72\) | \(t=0.72\) | \(t=5.57\) |
|     | \(F=1.71\) | \(F=4.00\) | \(F=5.17\) | \(F=5.17\) |

ZIDO-H\(^{\#}\)
(Capsules)

|                             |                   | 300 | 100.5±0.62 | 102.1±0.55 | 99.95±1.32 | 101.9±1.33 |
|                             |                   |     | Reference method | Titrimetry | Method A | Method B |
|                             |                   |     | \(t=4.32\) | \(t=0.90\) | \(t=2.27\) | \(t=4.60\) |
|                             |                   |     | \(F=1.27\) | \(F=4.53\) | \(F=4.53\) | \(F=4.60\) |

*Mean value of five determinations


Tabulated \(t\)-value at 95% confidence level is 2.77
Tabulated \(F\)-value at 95% confidence level is 6.39.
12.5 μg mL⁻¹ levels was determined by seven replicate analyses by titrimetric, spectrophotometric method A and method B, respectively. The relative error ranged from 0.67 to 1.82% and the RSD values were under 2%.

**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 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 of 95% confidence level. The calculated t- and F-values (Table 2) did not exceed the tabulated values (t=2.77, F=6.39) except in a couple of instances 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.

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. 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 experiment was repeated three times. The recovery of the pure drug added was quantitative (98.3-105.1%).

**Conclusion**

Three useful micro methods for the determination of ZDV have been developed and validated. The methods are simple and rapid taking not more than 15-20 min for the assay. The titrimetric method is the first ever proposed for zidovudine and is applicable over range 3 to 10 mg. Both the spectrophotometric methods are more sensitive than the existing UV and HPLC methods and are free from such experimental variables as heating or extraction step. The methods rely on the use of simple and cheap chemicals and techniques but provide a sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC. Thus, they can be used as alternatives for rapid and routine determination of bulk sample and tablets/capsules.

**References**