Quantitative methods for the assay of acyclovir in non-aqueous medium

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Three simple methods using visual titrimetric, potentiometric and spectrophotometric techniques are described for the determination of acyclovir in pure form and in pharmaceutical formulations. The methods are based on the neutralisation reaction involving the primary amino group of the drug and acetic perchloric acid in acetic acid medium. In titrimetric methods, the titration is completed with visual or potentiometric end-point detection, crystal violet being used as the indicator in visual titration. In spectrophotometry, the drug is treated with a fixed amount of perchloric acid-crystal violet mixture and absorbance of the resultant violet colour is measured at 570 nm and is related to drug concentration. Both titrimetric methods are applicable over 2-20 mg range of drug and the titration reaction follows a 1:1 stoichiometry. In spectrophotometry, Beer's law is obeyed over the concentration range 5-55 μg mL⁻¹ with an apparent molar absorptivity and Sandell sensitivity of 1.78 × 10⁴ L mol⁻¹ cm⁻¹ and 12.68 ng cm⁻², respectively. The limits of detection and quantification are calculated to be 1.696 and 5.654 μg mL⁻¹, respectively. The methods were successfully applied to the determination of acyclovir in tablets. The validity of the methods was further ascertained by parallel determination by a reference method and by recovery studies via standard-addition technique.

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Keywords: Acyclovir determination, potentiometry, titrimetry, spectrophotometry, antiviral drug

Acyclovir (ACL), 9-[2-hydroxyethoxy)methyl] guanine (Fig. 1), is an antiviral drug used extensively in the treatment of skin infections caused by herpes simplex virus¹. It is official in European Pharmacopoeia², British Pharmacopoeia³ and United States Pharmacopoeia⁴. The therapeutic importance of the drug has prompted the development of analytical methods for its assay. The most extensively used technique for the quantification of ACL in body fluids is high performance liquid chromatography (HPLC)⁵⁻¹⁹. The techniques such as radio immunoassay²⁰,²¹ high performance capillary electrophoresis²², liquid chromatography²³, and micellar liquid chromatography²⁴ are also confined to biological fluids including plasma and urine²⁰, plasma²¹,²³, urine²², serum and plasma²⁴. Chromatographic methods²⁵⁻²⁹ including HPLC²⁵⁻²⁷, HPLC-MS²⁸ and RPLC²⁹ have been applied for the determination of ACL in pharmaceutical formulations. Besides being tedious and difficult to perform, these procedures lack sensitivity. Methods based on derivative³⁰ and differential³¹ UV-spectrophotometry have also been reported for the assay of ACL in dosage forms. Recently³², a visible spectrophotometric method using Folin-Cioalteu reagent for the determination of ACL in pharmaceutical formulations has been reported. The present paper describes three simple methods based on neutralisation reaction. The titrimetric and visible spectrophotometric methods are the first to be developed for acyclovir based on neutralisation reaction. The proposed spectrophotometric method is more sensitive than the HPLC²⁷,²⁸ and derivative UV-spectrophotometric³⁰ methods reported previously.

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Experimental Procedure

Absorbance measurements were made on a Systronics model 106 digital spectrophotometer provided with 1-cm matched quartz cells. Potential measurements were made with a Systronics digital pH meter provided with combined glass-SCE electrode system, the KCl in the salt bridge being replaced with 0.1 M lithium perchlorate in glacial acetic acid. A 0.1 M perchloric acid was prepared by adding 2.1 mL of 70% HClO₄ (S.d.Fine Chem. India) to 5.2 mL acetic anhydride and diluting to 250 mL with glacial acetic acid in a calibrated flask, and kept overnight. The acid was standardised using pure potassium hydrogen phthalate and then diluted to 0.01 M by adding glacial acetic acid.

A 0.1% crystal violet indicator (S.d.fine Chem., India) was prepared by dissolving 117.6 mg of the reagent (85% dye content, S.d Fine Chem., India) in 100 mL glacial acetic acid.

For spectrophotometric work, perchloric acid-crystal violet mixture, equivalent to 1.5 mM HClO₄-0.25 mM crystal violet, was prepared by mixing 15.0 mL of 0.01 M HClO₄ and 10 mL of 1000 μg mL⁻¹ crystal violet solutions in a 100 mL calibrated flask and diluting to the mark with glacial acetic acid.

Pharmaceutical grade acyclovir was gifted by Cipla India Ltd., Mumbai, India and used as received. A stock solution containing 2 mg mL⁻¹ ACL was prepared by dissolving 500 mg of pure drug in 250 mL glacial acetic acid in a calibrated flask. This was appropriately diluted with glacial acetic acid to get 100 μg mL⁻¹ ACL for spectrophotometric work.

Tablets

Twenty tablets were weighed and ground into a fine powder. Tablet powder containing 200 mg of ACL was accurately weighted into a clean and dry 100 mL calibrated flask, 60 mL of glacial acetic acid added and shaken for 20 min. It is then diluted to the mark with glacial acetic acid. The contents were 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 filtrate was subjected to analysis by titrimetry with visual and potentiometric end-point detection. The tablet extract (2 mg mL⁻¹) was appropriately diluted with glacial acetic acid to get 100 μg mL⁻¹ solution for spectrophotometric analysis.

Analytical methods

Visual titration (Method A)

A 10 mL aliquot containing 2 - 20 mg of ACL was accurately measured into a 100 mL clean and dry titration flask, 2 drops of crystal violet indicator added and titrated with 0.01 M perchloric acid till the colour changed from violet to emerald green, The amount of the drug was calculated from the equation:

\[ \text{Amount (mg)} = \frac{VMR}{n} \]  \hspace{1cm} ... (1)

where, \( V \) = volume of perchloric acid consumed, mL; \( R \) = molarity of perchloric acid; \( M \) = molecular wt. of ACL; and \( n \) = number of moles of HClO₄ reacting with one mole of ACL.

Potentiometric titration (Method B)

An aliquot (30 mL) containing 2-20 mg of ACL was transferred into a 50 mL dry beaker. A modified glass-saturated calomel electrode was dipped in the solution. The solution was stirred magnetically and the titrant (0.01 M perchloric acid) was added from a micro burette. Near the equivalence point, the titrant was added in 0.2 mL increments, the solution was stirred for 30 s and steady potential was noted. The titration was continued until there was no significant change in the potential on further addition of the titrant. The equivalence point was located by the graphical method (Fig. 2). The amount of the drug was calculated using Eq. (1).

![Fig. 2—First derivative potentiometric titration curve for the titration of 10 mg of ACL with 9.3×10⁻³ M HClO₄](image-url)
Spectrophotometry (Method C)

Different aliquots (0.5-5.5 mL) of standard 100 μg mL⁻¹ ACL solid solution were delivered into a series of dry 10 mL calibrated flasks by means of a micro-burette. An exactly measured 2 mL volume of acid-dye mixture was added to each flask and the volume was made up to the mark with acetic acid, mixed well and the absorbance was measured at 570 nm. The absorbance values were plotted against the concentration of the drug to obtain the calibration graph. The concentration of the unknown was read from the calibration graph or calculated from the regression equation computed from the Beer’s law data.

Results and Discussion

Visual titrimetric and potentiometric methods are based on the determination of amount of drug by direct titration with perchloric acid. Spectrophotometry is based on the determination of excess of acid present after the reaction with ACL.

Method A

ACL is a weak base with one primary amine group, and neutralizes the added acid. The end-point was determined using crystal violet, the colour change from violet to emerald green being taken as the end point. Using 0.01 M perchloric acid, 2-20 mg of ACL can be conveniently determined. The reaction stoichiometry was found to be 1:1 which served as the basis for calculations.

Method B

Under the experimental conditions, ACL a weak base neutralizes the added perchloric acid and the end-point was indicated by the sudden jump in the potential (Fig. 2). The study shows that the stoichiometry of the reaction is 1:1 for the given range of 2-20 mg, with good accuracy and precision (Table 1).

Table I—Accuracy and precision

<table>
<thead>
<tr>
<th>Amount drug taken, mg</th>
<th>Amount drug found, mg</th>
<th>Relative error, %</th>
<th>RSD, %</th>
<th>Amount drug taken, mg</th>
<th>Amount drug found, mg</th>
<th>Relative error, %</th>
<th>RSD, %</th>
<th>Amount drug taken, µg</th>
<th>Amount drug found, µg</th>
<th>Relative error, %</th>
<th>RSD, %</th>
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</thead>
<tbody>
<tr>
<td>5.00</td>
<td>5.06</td>
<td>1.2</td>
<td>0.07</td>
<td>7</td>
<td>7.09</td>
<td>1.29</td>
<td>0.35</td>
<td>100</td>
<td>101.03</td>
<td>1.03</td>
<td>1.30</td>
</tr>
<tr>
<td>10.00</td>
<td>9.93</td>
<td>0.7</td>
<td>0.10</td>
<td>12</td>
<td>12.11</td>
<td>0.92</td>
<td>0.43</td>
<td>200</td>
<td>198.32</td>
<td>0.84</td>
<td>1.56</td>
</tr>
<tr>
<td>15.00</td>
<td>15.14</td>
<td>0.93</td>
<td>0.24</td>
<td>17</td>
<td>17.23</td>
<td>1.35</td>
<td>0.75</td>
<td>300</td>
<td>302.24</td>
<td>0.75</td>
<td>2.11</td>
</tr>
</tbody>
</table>

* Average of seven determinations; ** Average of three determinations

Method C

The method using crystal violet is based on the facts that the colour of the dye is controlled by the pH of the solution and that the colour change is not abrupt but occurs in a continuous manner over a definite range when the pH changes continuously. Crystal violet changes its colour from green at lower pH to violet at higher pH. When acyclovir is added in increasing amounts to a fixed amount of acid-dye mixture (green colour), the pH is progressively increased, and as a result the colour changes from green to violet, and the violet colour intensity increases. This is indicated by the proportional increase in the absorbance of the solution at 570 nm (Fig. 3) and is corroborated by the correlation coefficient, r = 0.9997.

In a preliminary study, 20 µg mL⁻¹ solution of crystal violet in acetic acid was found to produce a convenient maximum absorbance at 570 nm. This absorbance decreased to a constant minimum in the...
presence of 2 mL of 1.5 mM HClO₄ in a total volume of 10 mL. Hence, different amounts of drug were treated with a fixed amount of acid-dye mixture (emerald green in colour) to determine the concentration range of the drug that could be determined by the method of absorbance transitions of the dye accompanying pH changes. The dye colour was found to be stable for several hours in the presence of drug. Beer’s law was obeyed over 5 - 55 μg mL⁻¹. The apparent molar absorptivity was found to be 1.78×10⁴ L mol⁻¹ cm⁻¹ with a Sandell sensitivity of 12.68 ng cm⁻². The linear plot gave the regression equation: A = 0.0027+0.007 C (where A is the absorbance at 570 nm and C is the concentration of the drug in μg mL⁻¹ with a correlation co-efficient of 0.9997 (n = 11). The limit of detection and limit of quantification were 1.696 and 5.654 μg mL⁻¹, respectively.

Accuracy and precision of the method
Under the optimum conditions, the accuracy and precision of the proposed methods were determined by performing seven replicate analyses on pure drug solution at three levels. The results of the study presented in Table 1 are indicative of good accuracy and precision of the methods. From the results, it is

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Label</th>
<th>% found± SD</th>
<th>Reference method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acyvir DT tablet</td>
<td>200.00</td>
<td>99.06±2.22</td>
<td>101.32±1.75</td>
</tr>
<tr>
<td>Acyvir DT tablet</td>
<td>800.00</td>
<td>98.58±0.96</td>
<td>101.38±1.54</td>
</tr>
<tr>
<td>Ocuvir tablet</td>
<td>200.00</td>
<td>99.78±2.06</td>
<td>101.14±1.88</td>
</tr>
<tr>
<td>Ocuvir tablet</td>
<td>800.00</td>
<td>101.35±1.05</td>
<td>101.22±1.75</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3—Results of recovery studies by standard addition method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet</td>
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<tr>
<td>--------</td>
</tr>
<tr>
<td>Acyvir DT (200 mg)</td>
</tr>
<tr>
<td>Acyvir DT (400 mg)</td>
</tr>
<tr>
<td>Ocuvir (800 mg)</td>
</tr>
</tbody>
</table>

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* Average of five determinations in visual titrimetry, spectrophotometry and three determinations in potentiometry

# Tabulated value at 95% confidence level is 2.77 for visual titrimetry and spectrophotometry 2.37 for potentiometry.

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also clear that visual titrimetry is more precise and accurate than the potentiometric and spectrophotometric methods. Potentiometry is found to be more precise but less accurate than spectrophotometric method.

**Application**

The methods were applied to the determination of different brands tablets containing ACL. The results are tabulated in Table 2. The validity of the methods was tested by analyzing the same batch preparations by the reference method[27]. Statistical analysis of the results revealed that at 95% confidence level, the calculated t- and F-values did not exceed the tabulated values (Table 2) indicating that the proposed methods and the official method are comparable in accuracy and precision.

The accuracy and reliability of the methods were further ascertained through recovery studies. To a fixed and known amount of drug in the pre-analysed formulation, pure ACL was added at three different levels and the total was found by the proposed methods. The per cent recoveries of the added pure drug presented in Table 3 reveal that neither the end-point detection in titrimetry nor the absorbance measurements in spectrophotometry was affected by the commonly encountered excipients and diluents like talc, starch, sodium alginate, magnesium stearate, gum acacia, sucrose, calcium carbonate, calcium gluconate and calcium dihydrogen ortho phosphate.

**Conclusions**

The methods described which are based on the neutralisation of the amine in non-aqueous medium are simple, relatively specific, accurate and precise for the determination of ACL. Visual titrimetry is straightforward and rapid. The spectrophotometry employs mild working conditions without heating or extraction and is more sensitive than many HPLC procedures.

**Acknowledgement**

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**References**


