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Spectrophotometric Determination of Zidovudine in Pharmaceuticals Based on Charge-Transfer Complexation Involving N-Bromosuccinimide, Metol and Sulphanilic Acid as Reagents

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Abstract: A simple spectrophotometric method is proposed for the determination of zidovudine(ZDV) in bulk drug and in pharmaceutical preparations. The method is based on the oxidation of ZDV by a known excess of oxidant 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 purple-red reaction product absorbs maximally at 530 nm and Beer's law is obeyed over a range 5 to 75 µg mL^{-1}. The apparent molar absorptivity is calculated to be $5.1x10^3$ L $mol^{-1}cm^{-1}$, and the corresponding Sandell sensitivity value is 0.052 µg cm^{-2}. The limit of detection and quantification are found to be 0.90 and 2.72, respectively. Intra-day and inter-day precision and accuracy of the developed methods were evaluated as per the current ICH guidelines. The method was successfully applied to the assay of ZDV in tablet/capsule preparations and the results were statistically compared with those of the reference method by applying the Student's t-test and F-test. No interference was observed from the common tablet/capsule excipients. The accuracy of the method was further ascertained by performing recovery studies *via* standard-addition method.

Keywords: zidovudine, determination, spectrophotometry, N-Bromo-succinimide, pharmaceuticals.

Introduction

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. Zidovudine is a thymidine analogue[1]. It is phosphorylated in the body to zidovudine triphosphate which is the active form that inhibits HIV replication[2]. Zidovudine inhibits the key enzyme reverse transcriptase.

High performance liquid chromatography (HPLC) is the single most widely used technique for the determination of zidovudine, but most of the studies are devoted to the determination of drug in body fluids such as human serum [3-6], human plasma [7-12], human plasma and urine [13] and rat plasma [14]. There is only one report [15] on the use of HPLC for the specific determination of ZDV in pure drug and marketed tablets. UV-spectrophotometry has also found application in the simultaneous determination of ZDV and lamivudine in human serum[16]. The technique in different modes has been applied for the assay of ZDV when present alone[17] or in combination with lamivudine[18] in pharmaceuticals.

However, literature survey revealed that no visible spectrophotometric method has ever been reported for the determination of ZDV in pharmaceuticals. The present investigation aims to develop sensitive and cost-effective methods for the determination of ZDV in pure form and in tablet/capsule forms using spectrophotometric technique. Many pharmaceuticals[19-23] have previously been estimated spectrophotometrically using NBS as the oxidimetric reagent based on charge-transfer complexation reaction with metol and sulphanilic acid. The proposed method is based on the same reaction scheme and has the advantage of simplicity besides being accurate, and precise.

Experimental

Apparatus

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

Reagents and Standards

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

N-bromosuccinimide(1000 μg mL^{-1}): An appropriately 0.01 M solution was prepared by dissolving ~0.5 g of the chemical (SRL Research Chem., India) in hot water, filtered and diluted to 250 mL in a volumetric flask. The solution was standardized iodometrically[24] and was appropriately diluted to get a working concentration of 1000 μg mL^{-1} NBS. The solution was kept in a refrigerator when not in use.

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: Pharmaceutical grade zidovudine reported 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 1000 μg mL^{-1} ZDV was prepared, and was diluted appropriately with water to get 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.

Procedures

Bulk sample : Varying aliquots (0.5----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

A quantity of the finely ground tablet or 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 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.

Results and Discussion

Method development

One of the sensitive methods developed for the determination of primary aromatic amines involves the formation of purple-red colour when primary aromatic amines are made to react with metol and an oxidizing agent[25,26]. Of the several oxidizing agents employed for this study, NBS was found to be convenient because of its moderate oxidizing power. In the present paper, we describe the application of NBS-metol-primary amine combination to the determination of zidovudine .

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 amounts to a fixed amount 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 drugs.

The chemistry of the colour reaction may be suggested on the basis of a previously reported mechanism [27,28]. It is probable that the p-N-methylbenzoquinone monoimine 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 Figure 1.

The first step in this study was to fix the upper limit of NBS, which was found by treating different amounts 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 amounts of each drug were reacted with 1 mL of 1000 μg mL^{-1} NBS and the unreacted NBS was determined by following the general procedure.

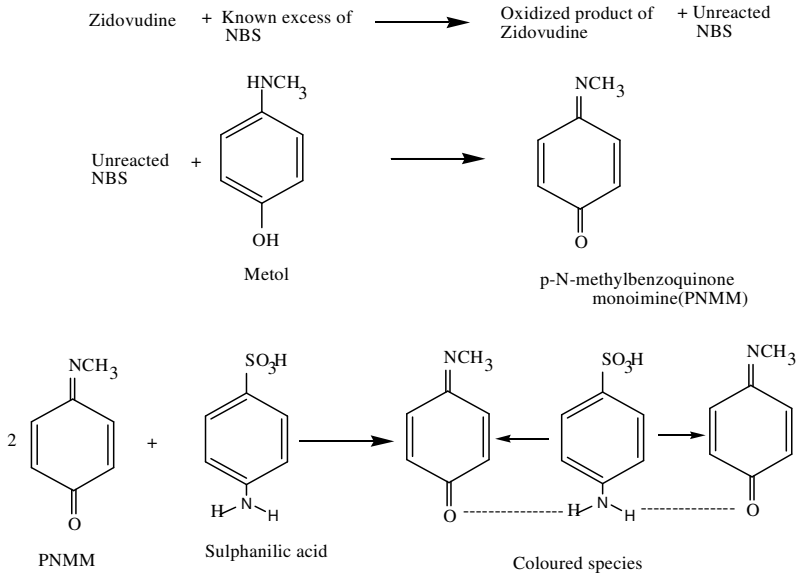

Figure 1. Probable reaction scheme

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 for both methods. The maximum colour intensity was 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.

Analytical data

A linear correlation was found between absorbance at λ_{max} and concentration of ZDV in the range given in Table 1. The graph (Figure 2) showed negligible intercept as 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 μg 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 1. The optical characteristics such as Beer's law limits, molar absorptivity and Sandell sensitivity values of both methods are also given in Table 1. The limit of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines[29] are also presented in Table 1 and reveal the very high sensitivity of the proposed method.

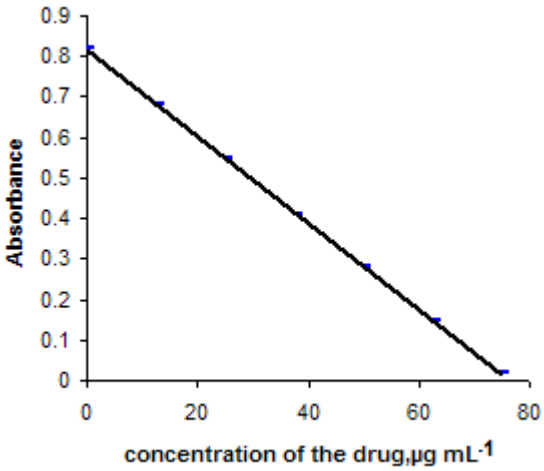

Figure 2. Linearity curve

Table 1. Analytical and regression parameters of proposed method.

Parameter	Method
λ_{max}, nm	530
Beer's law limits, (µg mL^{-1})	5.0 – 75.0
Molar absorptivity, (L mol^{-1} cm^{-1})	5.1×10^{3}
Sandell sensitivity, (µg cm^{-2})	0.0524
Limit of detection, (µg mL^{-1})	0.90
Limit of quantification, (µg mL^{-1})	2.72
Regression equation, Y*	
Intercept (a)	0.8113
Slope (b)	-0.011
Correlation coefficient, (r)	-0.9993
$S_{(a)}$	0.1929
$S_{(b)}$	0.009

*Y = a+bX, where Y is the absorbance and X concentration in µg mL^{-1}.
S_a= Standard deviation of intercept.
S_b= Standard deviation of slope.

Method Validation

To evaluate the accuracy and intra-day precision of the methods, pure drug solution at three different levels (amounts/concentrations) was analyzed, each determination being repeated seven times. The relative error (%) and relative standard deviation (%) were less than 2.5 and indicate high accuracy and precision of the methods (Table 2). For a better picture of reproducibility on a day-to-day basis, a series of experiments were performed in which standard drug solution at three different levels was determined each day for five days with all solutions being prepared afresh each day. The day-to-day relative standard deviation values were less than 3.0 % and represent the best appraisal of repeatability of the proposed methods.

Application to formulations: The validity of the method was checked by applying them to assay in two brands of capsules and tablets. Table 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 [17], 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 method is as accurate and precise as the reference method.

Table 2. Evaluation of accuracy and precision.

ZDV taken, (μg mL^{-1})	ZDV Found*, (μg mL^{-1})	Range, (μg mL^{-1})	RE (%)	SD, (μg mL^{-1})	SEM, (μg mL^{-1})	RSD, (%)	ROE,** (%)
12.5	12.35	0.37	1.18	0.17	0.06	1.36	±1.36
37.5	37.07	1.70	1.14	0.91	0.34	2.44	±2.44
62.5	61.67	1.30	1.33	0.76	0.29	1.23	±1.23

RE relative error; SD. Standard deviation; SEM .Standard error of mean;
RSD. Relative standard deviation; ROE. Range of error.
* Mean value of seven determinations
** At the 95% confidence level for 6 degrees of freedom.

Table 3. Results of determination of zidovudine in formulations and statistical comparison with the reference method.

Tablet/ Capsule Brand name[#]	Nominal amount, (mg)	Found* ± SD (%)			
		Reference method	Proposed method	t value	F value
VIRO-Z[a]	100	100.8±0.58	99.5±0.82	2.93	2.00
(Tablets)	300	99.50±0.36	100.3±0.45	3.12	1.56
ZIDO-H[b]	100	101.2±0.51	100.1±0.40	3.82	1.63
(Capsules)	300	100.5±0.62	99.1±0.55	3.78	1.27

*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

The accuracy and validity of the proposed method was further ascertained by performing recovery experiments. 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 such as talc, starch, gelatin, gum acacia, calcium carbonate, calcium gluconate, calcium dihydrogen orthophosphate, sodium alginate and magnesium stearate did not interfere in the determination.

Conclusions

The proposed method is simple, rapid and cost-effective. The proposed method is one of the most sensitive and is superior to the existing HPLC and UV-spectrophotometric methods. They rely on the use of simple and cheap chemicals, and inexpensive technique but provide a sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC. These advantages make the method highly suitable for routine use in laboratories as a part of industrial quality control.

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