



Research article

Determination of adefovir and strontium ranalate in bulk and formulations using methylene blue as chromogenic reagent

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Received: 15 Dec 2012 / Revised: 05 Jan 2013 / Accepted: 08 Jan 2013 / Online publication: 19 Jan 2013

ABSTRACT

Two simple, reproducible and sensitive spectrophotometric methods (A,B) were developed using methylene blue as chromogenic reagent to determine adefovir (ADE) and strontium ranalate (SRN) in bulk form or in pharmaceutical formulations. These methods are based on the formation of colored species by treating the drugs with chromogenic reagent methylene blue, which is extracted in chloroform and the drug concentration is measured at 650nm. The analytical parameters and their effects on the reported system were investigated. Beer's law was obeyed in the concentration ranges 5-35µg/mL (A), 4-20µg/mL (B). Molar absorptivity values were found to be 4.65x10⁴ (A), 1.53x10⁴ (B) and recoveries were found to be 99.80±0.56 to 99.98±0.66 (A), 99.55±0.77 to 100.32±0.44 (B), respectively. Interferences of the other ingredients and excipients were not observed. The proposed methods can be used successfully for the determination of ADE, SRN both in bulk and pharmaceutical formulations.

Key words: Spectrophotometry, Adefovir (ADE), Ion-association complex, Strontium ranalate (SRN)

1. INTRODUCTION

The chemical name of Adefovir (ADE) is 9-[2-bis[pivaloyloxy) methoxy]phosphinyl] methoxy]ethyl] adenine (Fig.1A). ADE is an acyclic nucleotide analog with activity against human hepatitis B virus (HBV). It is an acyclic nucleotide analog with activity against human HBV.

Strontium ranalate (SRN) is Distrontium 5-[bis(2-oxido-2-oxoethyl) amino]-4-cyano-3-(2-oxido-2-oxoethyl) thiophene-2-carboxylate (Fig.1B). SRN is the only anti osteoporotic agent which both increases bone formation and reduces bone resorption, resulting in a rebalance of bone turnover in favor of bone formation. SRN stimulates the calcium sensing receptors and leads to the differentiation of pre-osteoblast to osteoblast which increases the bone formation. SRN also stimulates osteoblasts to secrete osteoprotegerin in

inhibiting osteoclasts formed from pre-osteoclasts in relation to the RANKL system, which leads to the decrease of bone resorption. SRN is unusual in that the cation (strontium) is responsible for the pharmacological effect, whereas in most modern medications it is the base (anion) that is the active ingredient. In early scientific pharmacology, cations such as arsenic, bismuth, mercury and lithium were frequently used but recently anions have been much more in vogue.

A very few physio-chemical methods appeared in the literature for the determination of ADE [1-5], SRN by HPLC [6-7], AAS [8], spectrophotometric [9-10], flame photometry [11]. The analytically important functional groups of ADE are active methylene, purine, primary amine, phosphate ester were not properly exploited designing suitable spectrophotometric methods for the determination of the selected drug. The applications of methylene blue (MB) [12-20] as the chromogenic reagent are in good number and it is simplest reagent because of its well stability and the stability of colored complexes it formed with a series of reagents was

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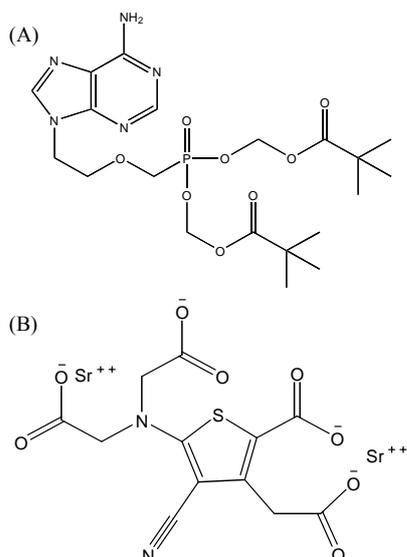


Fig.1. Chemical structure of (A) Adefovir-ADE (B) Strontium Ranalate-SRN

been well utilized by good number of researchers. It is clear from the literature that usage of MB for the determination of ADE, SRN the selected drugs by the author was not attempted.

Therefore in this research work, the author has made a valid attempt to develop two sensitive and reproducible methods for the assay of the ADE, SRN. The author has also developed simple and sensitive UV methods (0.1N HCl as solvent) and adopted it as a reference method for comparing accuracy of the results obtained by the proposed methods for ADE, SRN. ADE is official in [21-22] and SRN is official in [23-28].

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

All the chemicals and reagents were of analytical grade and the solutions were prepared freshly. MB Solution (Fluka, 0.01%, 3.12×10^{-4} M) was prepared by dissolving 10mg of methylene blue in 100mL of distilled water and washed with CHCl_3 to remove chloroformic soluble impurities. Buffer solution (pH 9.8) was prepared by mixing 7g of ammonium chloride with 56.8mL of liquor ammonia solution and diluted to 100mL with distilled water and pH was adjusted to 9.8. Chloroform (Qualigens) of AR grade was used.

2.2. Apparatus

A UV-1601 and SHIMADZU digital spectrophotometer with 1cm matched quartz cells were used for the spectral and absorbance measurements. A SYSTRONICS digital pH meter 361 was used for pH measurements.

2.3. Preparation of standard solutions

A 1mg/mL solution was prepared by dissolving 100mg of pure ADE in 100mL of water and further diluted to $50\mu\text{g/mL}$ - $400\mu\text{g/mL}$ (A), the stock solution (1mg/mL) of SRN was prepared by dissolving 100mg of it in minimum amount of 0.1N HCl and diluted to 100mL of distilled water. A portion of this stock solution was diluted stepwise with the same solvent to obtain the working standard SRN solution of concentrations of 40 - $200\mu\text{g/mL}$ (B).

Method A:

Aliquots of analyte (0.6-2.5mL, $80\mu\text{g/mL}$) is taken in a separation flask and then 1ml of buffer (pH = 9.8) and 5ml of MB are added and the volume is made up to 10mL. Then 10mL of chloroform is added and shaken well for 5 minutes. The organic layer is separated and the absorbance is measured each at 650nm (Fig.2). The concentration of drug is calculated from its Beer's plot (Fig.3).

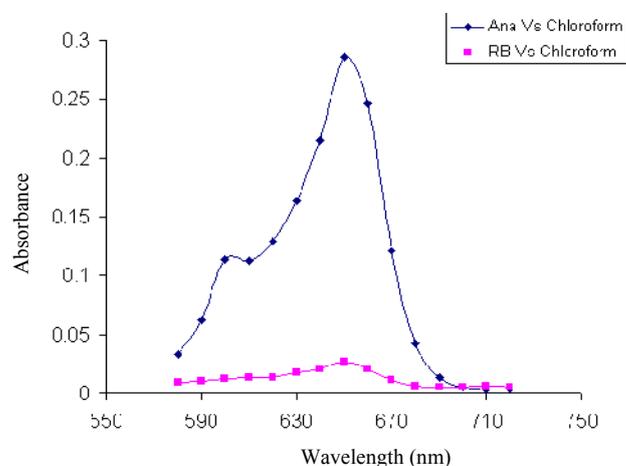


Fig.2. Absorption Spectrum of ADE with MB

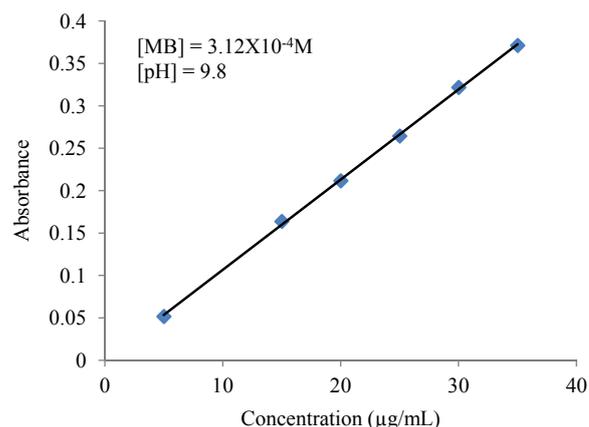


Fig.3. Beer's plot of ADE with MB

Method B:

Into a series of 125mL separating funnels containing aliquots of standard SRL solution [0.5-2.5mL, $80\mu\text{g/mL}$], 1mL of pH 9.8 buffer and 5.0mL of dye solution MB were added. The total volume of aqueous phase in each separating

funnel was adjusted to 10mL with distilled water and 10mL of CHCl_3 was added. The contents were shaken for 5min. The two phases were allowed to separate and the absorbances of the separated organic layer were measured at 650nm (Fig.4) against a similar reagent blank. The amount of SRN was deduced from the Beer's plot (Fig.5).

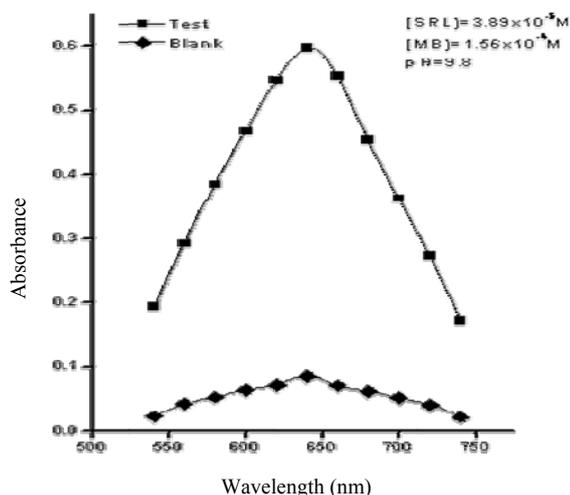


Fig.4.Absorption Spectrum of SRN with MB

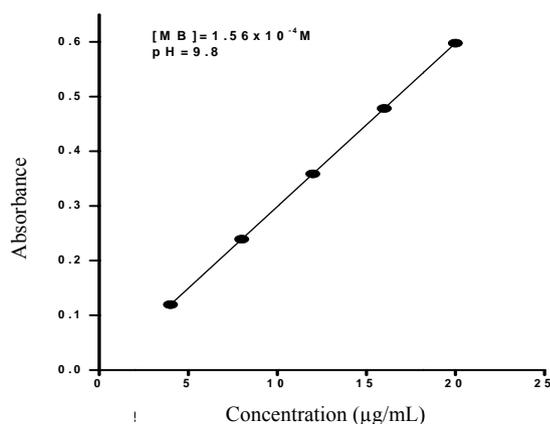


Fig.5.Beer's plot of SRN with MB

2.4. Preparation of formulation samples

Method A:

The tablet powder equivalent to 100mg of ADE was extracted with 3x25mL of chloroform and filtered. The combined filtrate was evaporated to dryness and the residue was dissolved in 100mL of distilled water to achieve a concentration of 1mg/mL stock solution. The solution was further diluted step wise with distilled water to get working standard solutions and analysed under procedures described for bulk samples.

Method B:

From one portion of chloroform extract (25mL), CHCl_3 was gently evaporated. The residue was dissolved in minimum amount of 0.1N HCl and diluted to 100mL of distilled water and subsequently brought the volume to 50mL with the same solvent to get 500 $\mu\text{g}/\text{mL}$. It was further diluted stepwise with the same solvent as described under standard solution preparation to obtain 80 $\mu\text{g}/\text{mL}$. Then the procedures given under bulk samples were followed for the assay of SRN in formulations.

3. RESULTS AND DISCUSSION

The optimum conditions for this method were established by varying one parameter at a time and keeping the others fixed and observing the effect produced on the absorbance of the coloured species. Beer's law limits, molar extinction coefficient, Sandell's sensitivity and regression characteristics of the method are presented in Table 1. The relative standard deviation and % range of error are also given in Table 1. Recovery studies were carried out by addition of known standard drug solution to pre analyzed sample solution. Results of recovery studies were presented in Table 2. The interference studies in the determination of ADE, SRN in pharmaceutical formulations revealed that the normally existing excipients and additives like hydroxyl propyl cellulose, lactose, carboxy methyl cellulose were found not to interfere even when present in excess. In developing the methods, a systematic study of the effects of various relevant parameters in the concerned were undertaken by varying one parameter at a time and controlling all other parameters to get maximum colour development, minimum blank colour, reproducibility and reasonable period of stability of final colored species formed.

Table 1

Optical characteristics of the proposed method

No.	Optical characteristics	A	B
1	λ_{max} (nm)	650	650
2	Beer's Law limits ($\mu\text{g}/\text{mL}$)	5-35	4-20
3	Molar absorptivity ($\text{L mol}^{-1}\text{cm}^{-1}$)	4.65×10^4	1.53×10^4
4	Correlation coefficient (r)	0.9995	0.9999
5	Sandell's sensitivity ($\mu\text{g}/\text{cm}^2/0.001$ absorbance unit)	0.0037	0.0334
6	Regression equation ($y=a+bc$)	0.0106	0.029
	(i) Slope (b)		
	(ii) Standard deviation on intercept (S_b)	1.261×10^{-4}	11.90×10^{-4}
	(iii) Intercept (a)	0.0001	0.00003
	(iv) Standard deviation (S_a)	3.001×10^{-3}	15.79×10^{-3}
	(v) Standard error of estimation (S_e)	3.046×10^{-3}	15.06×10^{-3}
7	Optimum photometric range ($\mu\text{g}/\text{mL}$)	15-35	9.33-19.95
8	Relative Standard deviation*	1.0102	0.488
9	Detection limit	0.849	0.512
10	% of range of error (confidence limit)	1.0603	0.804
	(i) 0.05 level		
	(ii) 0.01 level	1.7453	0.208

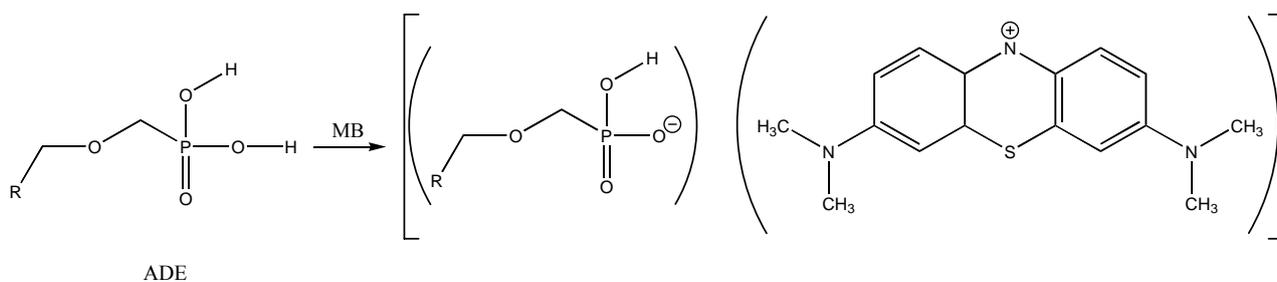


Fig.6.Colored Species of ADE/MB ion-association complex

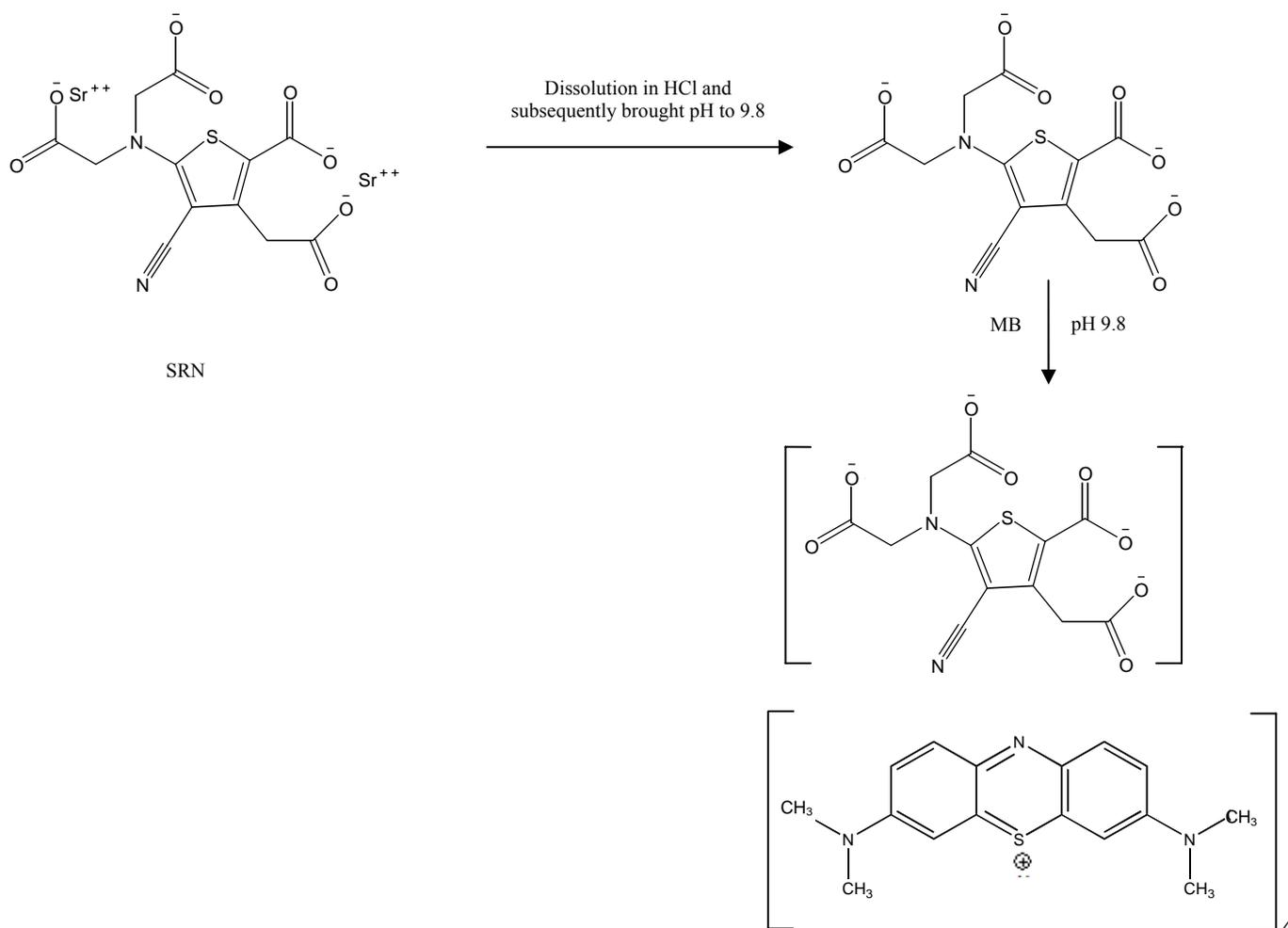


Fig.7. Colored species of SRN/MB ion-association complex

3.1. Nature of coloured species

Method A:

Adefovir (ADE) possesses different functional moieties such as active methylene, purine, primary amine, phosphate ester of varied reactivity. It is difficult to predict the exact nature of the existing coloured species of reasonable stability in each one of the proposed methods. Dye Stuff was used as analytical reagent. Cationic form of the dye MB, was involved in the formation of neutral coloured ion- association complex with negative charge (acid group in the drug) which

was extractable in to chloroform. An attempt has been made to indicate the nature of the coloured species in each proposed method for ADE based on analogy (reactive functional moiety in the drug, reagents, nature and probability, relative reactivities and impact of functional moieties one over the other) (Fig.6).

Method B:

As SRN possesses carboxylic acid in 4-oxo-3-quinoline carboxylic acid portion and it involves in ion association complex formation with a basic dye MB, which is extractable into chloroform from the aqueous phase. The anion form of

carboxylic acid (negative charge) of SRN molecule in alkaline pH medium is expected to attract the oppositely charged cationic form (positive charge) of the dye and behave as a single unit being held together by electrostatic force of attraction. The slope ratio of study revealed that the drug to dye mole ratio as 1:4. Based on analogy the structure of ion association complex was shown in scheme (Fig.7).

Table 2
Determination of ADE, SRN in pharmaceutical formulations

Sample	Label claim	Amount found by proposed method	Amount found by reference method	Percent recovery
Tablets – T ₁	200mg	99.94 ± 0.33 t = 1.64 F = 1.74	99.57 ± 0.25	99.75 ± 0.29
Tablets – T ₂	200mg	99.92 ± 0.23 t = 0.28 F = 1.32	99.92 ± 0.20	99.92 ± 0.22
Tablets – T ₃	200mg	99.80 ± 0.56 t = 0.60 F = 1.54	99.56 ± 0.45	99.66 ± 0.42
Tablets – T ₄	200mg	99.98 ± 0.66 t = 0.23 F = 1.74	99.83 ± 0.50	99.90 ± 0.38
Sachet – I	2mg	1.98±0.30 F=1.00 t=1.15	1.98 ±0.30	99.61 ±0.33
Granules - II	2mg	1.98±0.13 F=1.91 t=1.06	1.99 ±0.18	99.55 ±0.77
Sachet - III	2mg	1.98±0.30 F=1.33 t=1.00	1.97 ±0.26	100.16 ±0.49
Granules-IV	2mg	1.97±0.22 F=1.67 t=1.00	2.03 ±0.17	100.32 ±0.44

4. CONCLUSIONS

The proposed methods were superior in one way or other in terms of simplicity, λ_{\max} , ϵ_{\max} stability of coloured species over very few visible spectrophotometric methods reported so far. It can be seen from the results presented above, that the proposed methods have good sensitivity and λ_{\max} . Statistical analysis of the results (Table 1) shows that the proposed procedure has good precision and accuracy. Results of the analysis of pharmaceutical formulations reveal that the proposed methods were suitable for the analysis with virtually no interference of the usual additives. The proposed

methods are simple, sensitive, and reliable and can be used for routine determination of ADE, SRN in bulk samples and pharmaceutical formulations depending upon the needs of the specific situation.

ACKNOWLEDGMENTS

The author expresses his deep sense of gratitude to UGC, SAP, PHASE-III for its assistance.

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