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Spectrophotometric Determination of Salbutamol Sulphate and Acyclovir using Iron (III) and Ferricyanide

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Abstract : A simple, accurate, and cost-effective procedure for the estimation of acyclovir (ACL) and salbutamol sulphate (SBS) in bulk drug and in formulations has been developed. The method is based on the reduction of iron (III) by the studied drugs and subsequent interaction of iron (II) with ferricyanide to form Prussian blue. The product exhibitS absorption maximum at 760 nm. Beer's law is obeyed in the concentration ranges of 25-200 and 0.25-3.00 µ g ml^{-1} for ACL and SBS. respectively. The molar absorpthity and Sandell sensitivity values are 9.07 x 10^2 and 2.37 x 10^5 I mollcm.1 and 248.28 and 3.34 ng cm^{-2} for ACL and SBS, respectively. The limits of detection and quantification are reponed. Seven replicate analyses of the solutions containing three different concentrations of each substance were carried out and the percent error and the RSD values have been reponed. The proposed method was applied to the determination of the studied drugs in pharmaceutical formulations and the resuhs demonstrated that the method is equally accurate and precise as the official methods as found from the t- and F-values. The reliability of the method was established by recovery studies using standard-addition procedure.

Introduction

Acyclovir (ACL), [9-(2-hydroxyethoxy) methyl] guanine, whose structure is given in Fig 1 is an antiviral drug used extensively in the treatment of skin infections caused by herpes simplex virus.[1] It is official in European Pharmacopoeia,[2] British Pharmacopoeia[3] and United States Pharmacopoeia.[4] The therapeutic importance of this drug has prompted the development of analytical methods for its assay. The most extensively used technique for the quantification of ACL is high performance liquid chromatography (HPLC) but, most of the procedures using this technique are devoted to body fluids like plasma,[5-10] serum,[11-13] serum and urine,[14] and plasma and urine.[15] Even such techniques as radio immunoassay,[16,17] high performance capillary electrophoresis,[18] liquid chromatography,[19] and micellar liquid chromatography[20] are also limited to biological fluids including plasma and urine,[16] plasma,[17,19] urine,[18] serum and plasma.[20] Three methods including HPLC have been applied for the determination of ACL in pharmaceutical formulations[21-23] but, these procedures lack sensitivity with the concentration ranges being 0.1 to 1.0 mg ml^{-1} [22] and 50-200 µg ml^{-1} [23] besides being tedious and difficult to perform. Methods based on derivative[24] and differential[25] spectrophotometry have also been reported for the assay of ACL in dosage forms.

Salbutamol sulphate (SBS), whose structure is also given in Fig 1, is a selective beta-2-agoinst antiasthmatic. Its primary action is to stimulate adenyl cyclase which catalyses the formation of cyclic AMP. The cyclic AMP thus formed mediates smooth muscle relaxation and bronchodilation. Several techniques such as thin-layer chromatography,[26] HPLC,[27-31] titrimetry,[32] UV spectrophotometry[33,34] have been reported for the determination of SBS in pharmaceutical preparations. The most widely used technique for the assay of SBS has been visible spectrophotometry and procedures based on such varied reactions as nitration,[35,36] nitrosation,[37] nitrosation followed by chelation,[38] diazotisation[39-41] and coupling,[42] oxidative coupling[43,44] and reduction followed by chelation[45] have been reported. But, these methods lack sensitivity[38,45] require heating[35-37] and involve critical working conditions[43,44] and hence, are unsuitable for routine analysis.

The present work describes a simple, sensitive spectrophotometric method for the analysis of ACL and SBS. The method is based on the reduction of iron(III) by the studied drugs and subsequent interaction of iron(II) with ferricyanide to form Prussian blue.

Materials and Methods

Apparatus

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

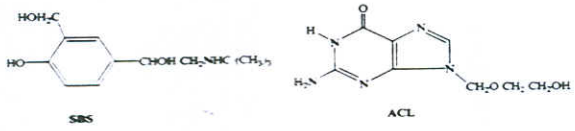

Fig 1. Structures of SBS and ACL

Reagents and solutions

All chemicals used were of analytical reagent-grade and double distilled water was used throughout the experiment unless specified otherwise. A 0.2% (W/V) solution each of anhydrous iron (III) chloride (NICE, India) and potassium ferricyanide (BDH, England) was prepared in water. 10 M sulphuric acid was prepared by adding 442 ml of concentrated acid (Sd Fine Chem., India Sp Gr, 1.83) to 558 ml of water with cooling.

Standard drug solution

Pure drug samples of ACL and SBS were gifted by Cipla India Ltd., Mumbai, and were used as received. Stock standard solution of ACL (2000 $\mu g\ ml^{-1}$) and SBS (100 $\mu g\ ml^{-1}$) were prepared by dissolving 200 mg of ACL and 10 mg of SBS, respectively, in water and diluting to 100 ml with water in separate volumetric flasks. The stock solutions were diluted appropriately to get the working standards of 1000 $\mu g\ ml^{-1}$ of ACL and 10 $\mu g\ ml^{-1}$ of SBS, with water.

Dosage forms

The following commercial formulations containing ACL were subjected to analysis by the proposed method: Acivir DT (200, 400 & 800 mg) and Ocuvir (200, 400 and 800 mg) tablets. Salbetol (2 and 4 mg), salmaplon (2 and 4 mg) tablets and ventrolin CR (4, 8 mg) capsules containing SBS were also analysed.

General procedure

Into a series of 10 ml of volumetric flasks, different aliquots (0.25 to 2.0 ml) of 1000 $\mu g\ ml^{-1}$ of ACL or 0.25-3.0 ml aliquots of 10 $\mu g\ ml^{-1}$ of SBS solution were transferred using a micro burette and the total volume was adjusted to 3.0 ml by adding water. Then, 3.0 ml each of ferricyanide (0.2%) and iron (III) chloride (0.2%) were added to each flask in the case of ACL, and for SBS, 2.0 ml each of iron (III) chloride and ferricyanide were added and mixed well and let stand for 5 and 10 min, respectively, for ACL and SBS. Finally, 1.0 ml of 10 M sulphuric acid was added to each flask and diluted to mark with water and mixed well. The absorbance of the resulting solution was measured at 760 nm against the reagent blank, prepared similarly. Calibration graph in each case was constructed by plotting the absorbance measured against the concentration of the drug. The concentration of the unknown was read from the respective calibration graph or calculated using the regression equation.

Procedure for dosage forms

About 20-40 tablets containing SBS or ACL were weighed and ground, and the powder equivalent to 100 mg each of SBS or ACL was transferred into a 100 ml volumetric flask, 60 ml water were added and shaken thoroughly for about 20 min and diluted to mark with water, and filtered. The filtrate containing SBS was further diluted to get the working concentration of 10 $\mu g\ ml^{-1}$. The general procedure was followed for the determination of ACL and SBS in tablet extracts. In the case of ventrolin CR capsules, the contents of 10 capsules were quantitatively transferred into a 100 ml volumetric flask and proceeded further as described under tablets.

Results and Discussion

ACL and SBS, being amines reduce iron (III) to iron (II). The latter, in turn, reacts with ferricyanide to form Prussian blue having the absorption maximum at 760 nm. Optimum conditions were established by variation of such parameters as iron (III), ferricyanide and acid concentrations, reaction time and order of addition of reactants.

Absorption spectra

Fig 2 shows the absorption spectra of the reaction products and the reagent blank. The product formed exhibited absorption maximum at 760 nm and the respective blanks display only slight absorption at this wavelength. Further, neither iron (III) nor ferricyanide solution absorbs at this wavelength. Hence, use of measured volumes of the reagent solution and

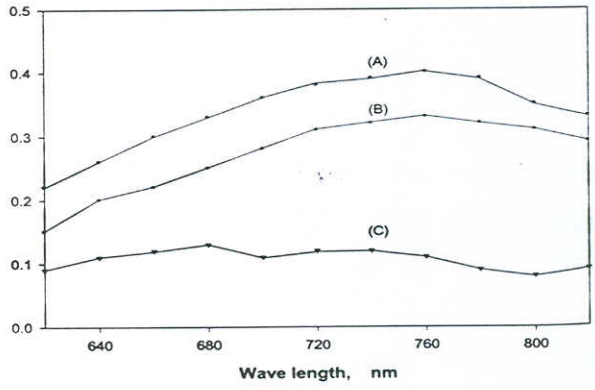

Fig 2. Absorption spectra of
(A). Reaction product of SBS (20 μg) with iron (III) – ferricyanide
(B). Reaction product of ACL (1000 μg) with iron (III) – ferricyanide
(C). Reagent blank

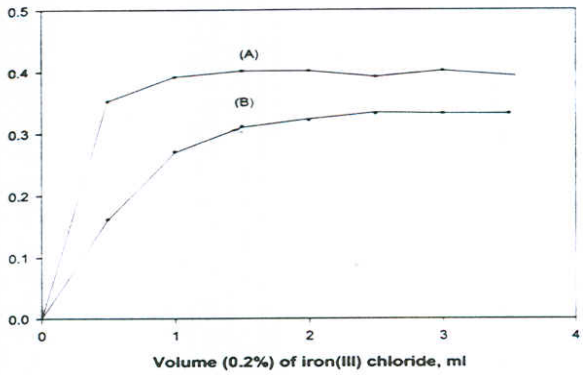

Fig 3. Effect of iron (III) chloride concentration (0.2%)
(A). SBS (20 μg) + 2.0 ml of 0.2% ferricyanide + 1.0 ml sulphuric acid per 10 ml.
(B). ACL (1000 μg) + 3.0 ml of 0.2% ferricyanide + 1.0 ml 10 M H_2SO_4 per 10 ml.

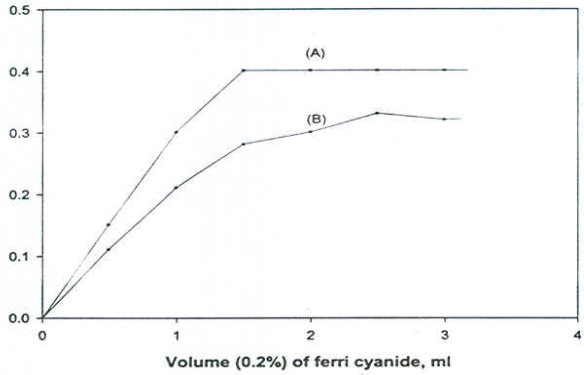

Fig 4. Effect of ferricyanide concentration (0.2%),
(A). SBS (20 μg) + 2.0 ml of 0.2% iron (III) chloride + 1.0 ml sulphuric acid per 10 ml
(B). ACL (1000 μg) + 3.0 ml of 0.2% iron (III) chloride + 1.0 ml 10 M H_2SO_4 per 10 ml

measurement against corresponding reagent blank gives the linear calibration graph for the drugs. The similarity of $\lambda_{max(s)}$ of the chromogen of both drugs suggests that the products formed have similar composition.

Effect of reagent concentration

A study of the effect of concentration of iron (III), ferricyanide and sulphuric acid with respect to maximum sensitivity and stability, minimum blank and obedience of Beer's law led to the general procedures described earlier. It was found that the addition of 2.5 ml of iron (III) chloride and 3.0 ml of ferricyanide solutions was necessary to obtain maximum absorbance in the case of ACL. And, 2.0 ml each of both the reagent solutions were found optimum in respect of SBS (Fig 3 and 4). However, 3.0 ml each of both the reagent solutions for ACL and 2.0 ml each for SBS were used in a total volume of 10 ml throughout the study to ensure complete reaction.

The reaction product, Prussian blue, was found to flocculate within 20-30 min of the colour development. To delay the flocculation, addition of acid after full colour development and before diluting to the mark was found necessary. Sulphuric acid was found to give more stable colour and reproducible product than hydrochloric acid. A 1.0 ml volume of 10 M sulphuric acid in a total volume of 10 ml was found adequate for the purpose.

Order of addition of reactants

After fixing all other parameters, a few other experiments were performed to ascertain the influence of the order of addition of reactants. The order: drug, ferricyanide and iron (III) followed by sulphuric acid after full colour development gave maximum absorbance and stability, and hence the same order of addition was followed throughout the investigation.

Effect of reaction time and stability of coloured species

Reaction is slow at room temperature (32±2 °C) but the absorbance increases with time and reaches a maximum in 10 min for both drugs. The developed colour remained stable for at least 2 h for ACL and 6 h for SBS.

Analytical appraisal

Under the experimental conditions described, Beer's Law is obeyed over the concentration ranges of 25-200 and 0.25-3.0 μg ml^{-1} of ACL and SBS, respectively, except in the vicinity at the origin. The molar absorptivities at 760 nm are 9.07×10^2 and 2.37×10^5 l $mol^{-1}cm^{-1}$ for ACL and SBS, respectively. The calculated Sandell sensitivities are 248.28 and 3.34 ng cm^{-2} for ACL and SBS, respectively. The limits of detection are 5.120 and 0.039 μg ml^{-1} for ACL and SBS, respectively, and the limits of quantification are calculated to be 17.050 and 0.130 μg ml^{-1} for ACL and SBS, respectively.

The equations relating absorbance to concentration calculated using the method of least squares are:

$$A_{760} = 0.108 + 2.176 \times 10^{-3}\ C \text{ for ACL}$$
$$A_{760} = 0.103 + 0.152\ C \text{ for SBS}$$

where A = absorbance at 760 nm, C = concentration in μg ml^{-1}.

The correlation coefficients (r) are 0.9997 (n=8) and 0.9998 (n=7) for ACL and SBS, respectively, indicating good linearity.

Accuracy and precision

Accuracy and precision were established by performing seven replicate determinations containing different amounts within the Beer's law limits. The

Table 1. Accuracy and precision.

Drug	Amount taken µg	Amount found* µg	Range, µg	Error, %	SD, µg	RSD, %
ACL	500.00	491.45	1.50	1.71	1.05	0.21
	1000.00	1009.00	0.75	0.90	1.55	0.15
	1500.00	1491.60	1.01	0.56	0.88	0.06
SBS	5.00	4.97	0.13	0.60	0.06	1.18
	15.00	15.13	0.52	0.87	0.19	1.29
	25.00	24.97	0.34	0.12	0.41	1.63

* Average of seven determinations.
▾ t is tabulated value (2.447) at 95% confidence level, n=7 and s is standard deviation.

Table 2. Results of determination of ACL and SBS in dosage forms.

Drug and Maker*	Nominal amount/mg	Drug found▾, mg	Error, %
ACL			
Acivir DT[a] tablets	200.00	202.75	0.38
	400.00	396.39	0.90
	800.00	808.85	1.11
Ocuvir[b] tablets	200.00	197.68	1.16
	400.00	403.30	0.83
	800.00	789.69	1.30
SBS			
Salbetol tablets[b]	2.00	2.01	0.57
	4.00	4.07	1.75
Salmaplon tablets[b]	2.00	1.99	0.50
	4.00	4.08	2.00
Ventrolin CR capsules[c]	4.00	4.03	0.71
	8.00	7.99	0.13

* Marketed by a. Cipla, b. FDC, c. Glaxo Allendrugs India.
▾ Average of five determinations.

range, percent error, standard deviation, relative standard deviation (RSD, %) for seven determinations at each level are given in Table 1. The accuracy of the method is evident from the percent error which is less than 2 and 1% for ACL and SBS, respectively. The RSD values which are less than 2.0% for three different levels of drugs, indicate the high reproducibility of the method.

Application

The method was applied to the determination of ACL and SBS in proprietary drugs purchased from local stores and containing other inactive ingredients. The results in Table 2 show that the method is successful for the determination of ACL and SBS and that the excipients in the dosage forms do not interfere. A statistical comparison of the determination of ACL and SBS by the proposed method and official methods[3,46] for the same batch of materials is presented in Table 3. The Student's t- and F-values indicate that there is no significant difference between the methods in respect of accuracy and precision.

To study the reliability and reproducibility of the proposed method, standard-addition method was followed. A fixed amount of each drug from preparations was taken and pure (standard) drug at three different levels was added. Each determination was repeated three times. The total amount was found by the proposed method and the percent recovery of the added standard was calculated from:

$$\% \text{ recovery} = [\, C_v - C_u / C_a \,] \times 100$$

where,

C_v = total concentration of the analyte measured
C_u = concentration of the analyte present in the formulation
C_a = concentration of the analyte (pure drug) added to formulation

Results of this study presented in Table 4. reveal that the method was unaffected by the various excipients present in the formulations.

Table 3. Comparison of results of ACL and SBS determination by the proposed method with those of official method

Preparation	Found* (% Recovery ± SD)		t-value (2.776)#	F-value (6.39)#
	Proposed method	Official method		
ACL				
Acivir DT tablets (200 mg)	100.38±0.45	99.86±0.35	2.05	1.65
Acivir DT tablets (400 mg)	100.10±0.54	100.90±0.45	2.56	1.44
Ocuvir tablets (800 mg)	99.96±0.38	100.06±0.18	0.56	4.46
SBS				
Salbetol tablets (2.0 mg)	100.50±0.09	100.35±0.15	1.98	2.78
Salmaplon tablets (4.0 mg)	102.00±0.13	101.16±0.22	1.44	2.86
Ventrolin CR capsules (8.0 mg)	99.88±0.15	100.21±0.25	2.61	2.78

* Mean value of five determinations.
\# Figures in parentheses are the tabulated values at 95% confidence level.

Table 4. Results of recovery study by standard –addition method.

Formulations	Amount of drug in formulation, mg	Amount of pure drug added, mg	Total found*, mg	% recovery of pure drug added
Acivir DT tablets (200 mg)	250.95	500.00	734.80	96.85
	250.95	1000.00	1190.99	94.00
	250.95	1500.00	1794.37	102.89
Ocuvir tablets (400 mg)	249.90	500.00	739.33	97.89
	249.90	1000.00	1239.65	98.98
	249.90	1500.00	1766.52	101.11
Salbetol tablets (4.0 mg)	2.51	5.00	7.43	98.40
	2.51	15.00	17.63	100.80
	2.51	25.00	27.01	98.00
Ventrolin CR capsules (8.0 mg)	2.49	5.00	7.82	104.60
	2.49	15.00	17.32	98.87
	2.49	25.00	27.18	98.76

* Mean value of three determinations

Conclusion

The procedure described in this paper is simple and does not need the elaborate treatment and tedious extractions required for chromatographic methods. Also, the method is more sensitive than many spectrophotometric methods reported for either drug.

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