CONDENSATION, CHARGE TRANSFER AND ION ASSOCIATION COMPLEX FORMATION REACTIONS FOR THE SPECTROSCOPIC DETERMINATION OF SPIRAMYCIN IN PURE AND PHARMACEUTICAL FORMULATION

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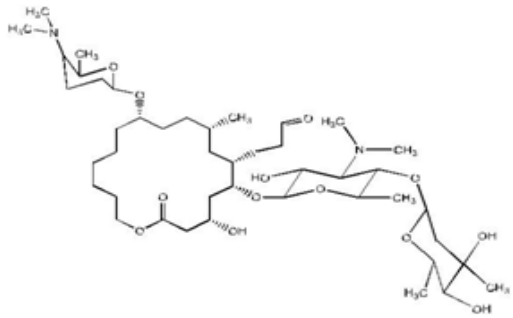
ABSTRACT
Eight simple, accurate and more sensitive spectrophotometric methods have been developed for the determination of Spiramycin (SPI), in both pure and pharmaceutical preparations. The method M1 p-dimethylaminobenzaldehyde (PDAB) and M2 Vanillin are condensation reactions with Spiramycin. The method M3 Chloranil and method M8 2,6-dichloroquinone N-chlorimide (DCQC) forms charge transfer complex with SPI. The method M4 wool fast blue (WFB) and M5 Bromo Cresol Green (BCG) involves ion association complex formation with Spiramycin. The method M6 Folin-Ciocalteu reagent (F-C Reagent) the color formation with Spiramycin is due to oxidation - reduction and method M7 Citric acid/Ac2O forms colour complex with Spiramycin. Regression analysis of Beer's law plots showed good correlation in the concentration range of 5.0 - 50, 5.0 - 50, 2.5 - 15, 2.5 - 15, 5.0 - 30, 2.5 - 10, 2.5 - 15 and 2.5 - 15 and the corresponding molar absorptivity values are 1.4247 x 10^4, 1.256 x 10^4, 4.324 x 10^4, 5.4967 x 10^4, 6.1543 x 10^3, 7.0226 x 10^4, 5.1594 x 10^4 and 3.389 x10^4 for methods M1, M2, M3, M4, M5, M6, M7 and M8 respectively. All variables have been optimized and the results were statistically compared with those of literature methods by employing the student's T-test and F-test. No interference was observed from excipients normally added to the tablets.

Keywords: *Spiramycin (SPI), Visible spectrophotometry; Vanillin, Chloranil, p-Dimethyl amino benzaldehyde*

INTRODUCTION

Spiramycin(SPI) is the antibiotic drug, white or faintly yellowish powder which is insolublein water, soluble in acetonitrile, acetone, and methanol. Its melting point is 128 - 137^{0}C and pH 8.5 – 10.5 (0.5% solution). It is 16 membered ring macrolide. SPI was discovered in 1952 as a product of Streptomyces ambofaciens. An important part is played by the slow release of the antibiotic from the tissue compartment, the marked action on microbes in sub-inhibition concentrations and the relatively long persisting post-antibiotic effect. It is available for parenteral and oral administration.



Spiramycin

(4*R*,5*S*,6*R*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-10-{[(2*R*,5*S*,6*R*)-5-(dimethylamino)-6-methyltetrahydro-2*H*-pyran-2-yl]oxy}-9,16-dimethyl-5-methoxy-2-oxo-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-6-yl 3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-3-(dimethylamino)-α-D-glucopyranoside

Quality assurance and control of pharmaceutical chemicals and formulations is essential for assuring the availability of safe and effective drug formulations to consumers. Quantitative estimation of chemical entity of a drug is vital for maintaining and assuming the quality. The survey on the existing analytical methods reveals that relatively little attention was

paid to the development of visible spectrophotometric methods for SPI. Electrochemical study[1], HPLC [2], capillary electrophoresis[3], Potentiometric[4], voltammetry[5], liquid chromatograph[6,] LC–MS/MS [7], HPLC[8], reversed-phase liquid chromatographic[9], HNMR[10], C13NMR[11] and spectrophotometric techniques[11,12,13] were available in literature for the estimation of drug. The chemical features of the drug molecule offers a lot of scope for the development of new methods with better sensitivity, specificity, precision and accuracy. The reported chromatographic techniques (HPLC or GC) require expensive experimental set-up and are not affordable in every laboratory for routine analysis. Although visible spectrophotometric methods are the instrumental methods of choice commonly used in industrial laboratories, for their simplicity, selectivity and sensitivity. Therefore, the need for a fast, low cost and selective method is obvious, especially for routine quality control analysis of pharmaceutical products containing Spiramycin. This paper describes the development of sensitive and rapid spectrophotometric methods by the condensation reaction of spiramycin with p-dimethylaminobenzaldehyde (PDAB) (M1) and Vanillin (M2); charge transfer complex reaction with Chloranil (M3) and 2,6-dichloroquinone N-chlorimide (DCQC) (M8); ion association reaction with the dyes wool fast blue (WFB) (M4) and Bromo Cresol Green (BCG) (M5); complex formation with Citric acid/Ac2O (M7) and oxidation reduction reaction with Folin-Ciocalteu reagent (F-C Reagent) (M6). All these methods have been found to be satisfactory for the determination of Spiramycin in pure and pharmaceutical formulations.

MATERIALS AND METHODS

All spectral and absorbance measurements were made on a Systronics 106 model visible Spectrophotometer with 1 cm matched quartz cells. An Elico 120 digital pH meter was used for pH measurements.

Reagents

All chemicals and reagents used were of analytical reagent grade and distilled water was used throughout the investigation.

Standard SPI solution

Accurately weighed 100 mg of pure or pharmaceutical preparation (tablet was dissolved in acetic acid), filtered through Whatman No. 41 filter paper, the filtrate was made to 100 ml with glacial acetic acid. 10 ml of this solution was taken and made to 50 ml with glacial acetic acid to get a concentration of 200 µg/ ml.

Method M1

PDAB solution (Reachim, USSR 0.4%, 2.68 x 10-2M): Prepared by dissolving 400 mg of P-dimethyl aminobenzaldhyde in 100 ml glacial acetic acid.

Method M2

Vanillin solution (BDH, 0.4%, 2.63 x 10-2M): Prepared by dissolving 400 mg of Vanillin in 100 ml glacial acetic acid.

Method M3

Chloranil solution (BDH; 0.1%, 4.0 x 10^{-3}M): Prepared by dissolving 100 ml of chloranil in 100 ml of methanol.Buffer (pH 7.0): Prepared by mixing 61.2 ml of Na2HPO4 (0.067M) and 38.8 ml KH2PO4(0.067M) and pH of the solution was adjusted to pH 7

Method M4

WFB solution (Fluka: 0.2% w/v, 3.26 x 10^{-3} M): Prepared by dissolving 200 mg of wool fast blue in 100 ml of distilled waterGlycine buffer solution (pH 1.5): Prepared by mixing 289 ml of glycine solution (37.52 g of glycine and 29.24 g of NaCl were dissolved in 500 ml of distilled water) with 711 ml of 0.1M HCl and pH of the solution was adjusted to 1.5.

Method M5

BCG solution: prepared by adding 99.8 mg of BCG to 2 ml of 0.1M NaOH and 20 ml of ethanol, making upto 100 ml with distilled water. Buffer (pH 4.0): Prepared by dissolving 40.846 g of KHPO4in 100ml distilled water and 408 ml of 0.1 N HCl are mixed and brought to 200 ml withwater.

Method M6

F-C Solution (Loba 2.0N): Folin-Ciocalteu reagent supplied by Lobachemie was used in the investigations directly.Na2CO3 (BDH; 10%, 9.43x10^{-1} M or 30% 2.83M): Prepared by dissolving 10.0g of Na2CO3 in 100 ml of distilled water.

Method M7

Citric acid monohydrate (BDH;3%, 0.72 x

10^{-2}M): Prepared by dissolving 7.5 g of citric acid monohydrate in 250 ml of acetic anhydride

Method M8

DCQC solution Loba; 0.05%, 2.38 x 10^{-3}M w/v): Prepared by dissolving 50mg of DCQC in 100 ml of isopropanol.

EXPERIMENTAL

Preparation of solutions

Method M1 (PDAB)

To each one of 10 ml calibrated tubes, aliquots of (0.25-2.5 ml of 200 µg/ml) SPI,solution; 2.0 ml of 2.68 x $l0^{-2}$M PDAB and 3.0 ml of concentrated hydrochloric acid were added successively and the total volume in each flask was brought to 7.5 ml by the addition of glacial acetic acid and kept aside for 5 min. Then the flasks were made up to the mark with glacial acetic acid and the absorbance was measured at 480 nm against a reagent blank after 10 min.

Method M2 (vanillin)

To each one of 10 ml calibrated tubes, aliquots of SPI, solution (0.25-2.5 ml of 200 µg/ml); 2.0 ml of 2.63 x $l0^{-2}$M Vanillin and 3.0 ml of concentrated hydrochloric acid were added successively and the total volume in each flask was brought to 7.5 ml by the addition of glacial acetic acid and kept aside for 5 min. Then the flasks were made up to the mark with glacial acetic acid and the absorbance was measured at 500 nm against a reagent blank after 10 min.

Method M3 (chloranil)

To each one of 10 ml calibrated tubes, aliquots of standard drug solution (0.5 – 2.0 ml, 50 µg/ml) of SPI was transferred, 4 ml of (pH 7) buffer, and 1.0 ml of chlorani1 (4.0 x10^{-3}M) solution was added and made upto 10 ml with distilled water. The absorbance of the colored species was taken at 520 nm for SPI is observed against reagent blank after 10 min.

Method M4 (WFB)

Aliquots of standard SPI solution (0.5 – 3.0 ml, 50 g/ml) were transferred in a series of 125 ml separating funnels. 1.0 ml buffer solution (pH 1.5) and 0.5 ml of WFB were added respectively. The total volume of aqueous phase in each separating funnel was adjusted to 10.0 ml with distilled water and 10 ml of chloroform was added to each separating funnel and the contents were shaken for 2 minutes and allowed to separate. The two phases were allowed to separate and the chloroform layer was passed through anhydrous sodium sulphate and the absorbance was measured immediately at 590 nm against a reagent blank.

Method M5: (BCG)

Aliquots of the standard SPI solution (0.5 – 3.0 ml, 100 g/ml) were transferred into a series of 125 ml separating funnels. 1 ml of potassium hydrogen phthalate buffer (pH 4) and 1 ml of 0.1 % BCG were added. The total volume of aqueous phase in each separating funnel was adjusted to 10.0 ml with distilled water and 10 ml Chloroform was added to each separating funnels and then the contents were shaken well and left at room temperature for 5 minute and allowed to separate. The two phases were allowed to separate and the chloroform layer was passed through anhydrous sodium sulphate. The absorbance of yellow coloured complexes is measured at 410 nm against the corresponding reagent blank.

Method M6 (F-C reagent)

To each one of 10 ml calibrated tubes, aliquots of the standard SPI solution (0.5 - 2.0 ml, 50 µg /ml) were transferred. To each tube 2 ml of 2N F-C reagent and 5.0 ml of 2.83M Na2CO3 was added, kept aside for 10 min at room temperature. The solution was diluted to the mark with distilled water. The absorbance of the solutions was measured at 770 nm against the reagent blank after 10 min.

Method M7 (citric acid - acetic anhydride reagent)

Aliquots of standard SPI solution (0.5 - 3.0 ml, 50 µg /ml) were taken into a series of 25 ml graduated tubes and gently evaporated on a boiling water bath to dryness. To this 10 ml of citric acid - acetic anhydride reagent was added and the flasks were immersed in a boiling water bath for 30 min. The tubes were cooled to room temperature and made up to 10 ml with acetic anhydride. The absorbance of the colored solutions was measured after 15 min at 570 nm for SPI against reagent blank.

Method M8 (DCQC)

Aliquots of SPI solution (0.5 - 3.0 ml, 50 µg/ml) of were transferred a series of 25-ml

calibrated; to each tube 2.0 ml of DCQC solution were added successively and the total volume in each was brought to 10.0 ml with Isopropanol. The absorbance was measured at540 nm for SPI against a reagent blank after 10 min.

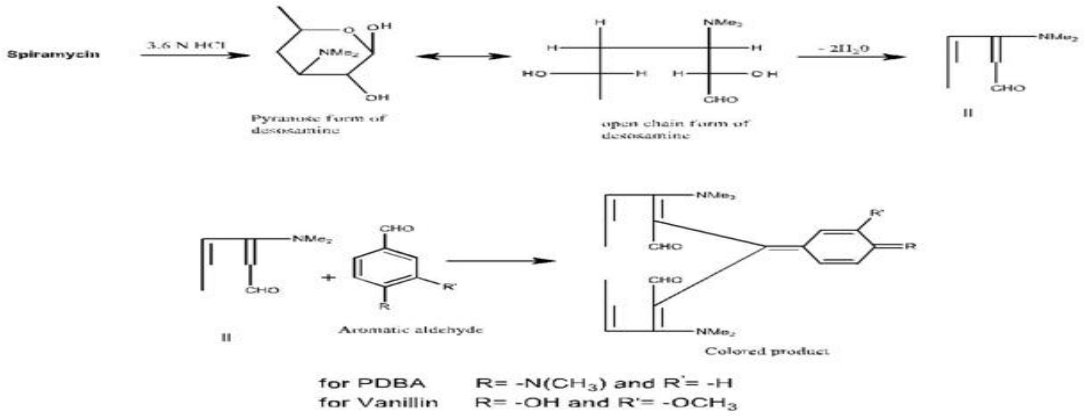
Preparation of SPI

Accurately weighed 100 mg of pure or pharmaceutical preparation (tablet was dissolved in 20.0 ml methanol and filtered to remove the insoluble portion (if any) , the filtrate was made upto 100 ml with methyl alcohol (1 mg/ml). The final concentration of SPI was brought upto 100 µg/ml with methyl alcohol and mixed well and filtered using a Whatman No.41 filter paper. An appropriate dilute solution was subjected to analysis by the procedures described above.

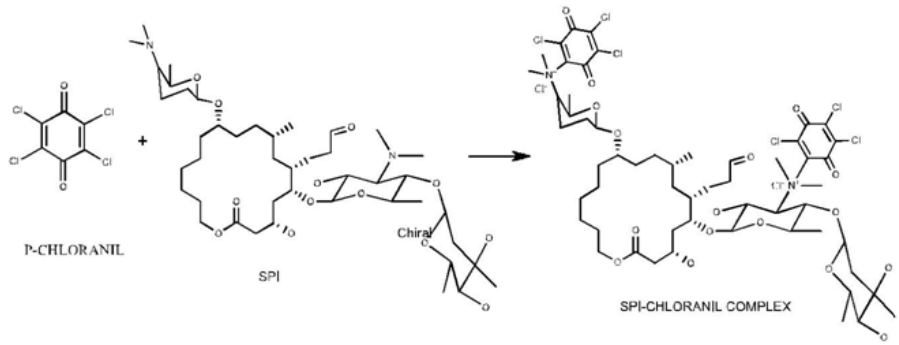
RESULTS AND DISCUSSION

The formation of the colored complex with SPI in condensation reaction of aromatic aldehyde vanillin (M1) or PDAB (M2) furnishing the corresponding colored product is presented in Scheme-1. The charge-transfer color complex formation of Chloranil (M3) and SPI is shown in Scheme-2. The tertiary amine in SPI involves in ion association complex formation with an acidic dye WFB (M4) the structure of complex is shown in Scheme -3. It was observed that the anionic dyes such as BCG (M5) also form ion-association complex with the drug SPI to give complex, the structure of complexes are shown in Scheme - 4. The active constituent of F-C reagent is phosphomolybdic tungstic mixed acid. The mixed acids in Folin-Ciocalteu preparation are the final chromogen and involve the following chemical species.

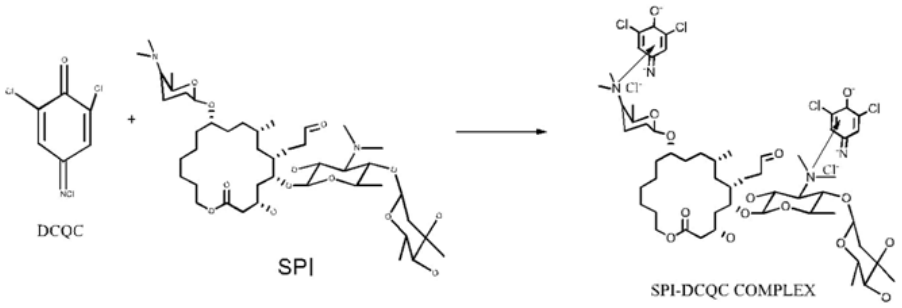
3H2O. P205. 13 WO3. 5 MoO3. 10 H2O and 3H2O. P2O5. 14 WO3. 4 MoO3. 10 H2O SPI probably effects a reduction of 1, 2 or 3 oxygen atoms from tungstate and/or molybdate in F-C reagent (M6) with characteristic intense blue color. The SPI permits the developmentof a complex with Citric acid - Acetic anhydride (Method M7) which is shown in Scheme- 5. DCQC forms C-T complex with SPI containing basic nitrogen resulting colour is shown in scheme-6.



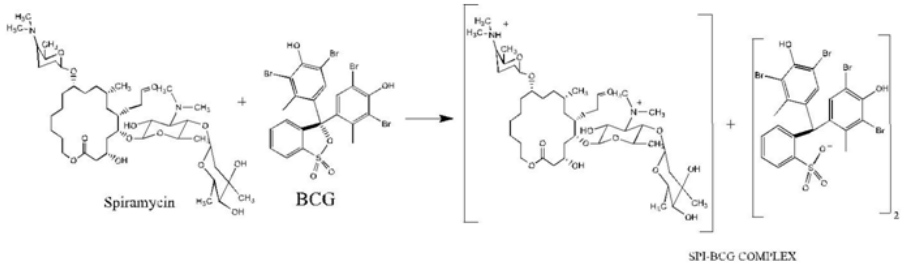
Scheme – 1: PDBA and Vanillin forming colour complex with SPI



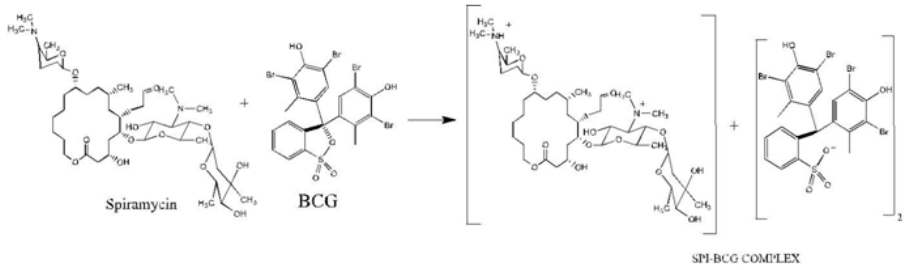
Scheme - 2: Charge-Transfer colour complex of SPI –Chlorinil



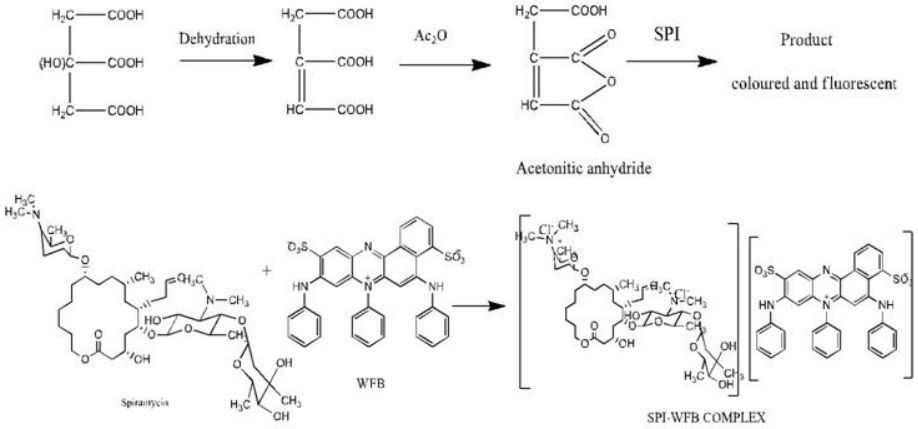
Scheme - 3: Ion association complex of SPI – WFB



Scheme - 4: Ion association complex of SPI – BCG



Scheme - 5: Coloured complex of SPI - Citric acid - Acetic anhydride



Scheme – 6: Charge-Transfer colour complex of SPI - DCQC

Method - M1 (PDAB) and Method - M2 (vanillin)

In order to ascertain the optimum wavelength (λmax) of the colored product formed on treating the SPI with PDAB or with vanillin, the absorption spectrum was scanned in each case on a spectrophotometer in the wavelength region of 360 - 700 nm with various parameters such as concentration and volume of vanillin or PDAB, volume of acid, order of addition of reagents, solvent for final dilution against a reagent blank and the results are graphically presented (Fig. 1 and Fig. 2). The absorbance curve shows characteristic absorption maximum at 480

nm and 500nm Method M1 method M2 respectively where as PDAB or vanillin have low or no absorption in this region

Method - M3

The absorption spectra of the resulting colored species obtained by treating a fixed quantity of SPI with the chloranil reagent was scanned in the wavelength region of 400 - 800 nm with the effect of various parameters like volume of chloranil, effect of pH, nature and volume of buffer, effect of temperature, effect of solvent for final dilution, stability of the colored species against a reagent blank and the results are presented graphically in (Fig. 3). The wavelength of maximum absorbance was found to be 520 nm whereas the blank in thismethod (omitting drug) have low or no absorption in this region.

Method - M4

In order to ascertain the optimum wavelength (λmax) for rapid and quantitative formation of the colored species with maximum stability and sensitivity formed on mixing the drug (SPI) with WFB the absorption spectrum in each case was scanned on a spectrophotometer in the wavelength region 360 - 800 nm for a series of solutions against a reagent blank varying one and fixing the other parameters in each case such as type and volume of acid or type and volume of buffer, concentration of dye, organic solvent used for extraction, ratio of organic phase to aqueous phase during extraction, shaking time and temperature and the results are graphically presented (Fig. 4).

Method - M5

In order to ascertain the optimum wavelength (λmax) for rapid and quantitative formation of the colored product with maximum stability and sensitivity formed on mixing the drug (SPI) with BCG the absorption spectrum in each case was scanned on a spectrophotometer in the wavelength region 360 - 800 nm a series of solutions, varying one and fixing the other parameters in each case such as type and volume of buffer, concentration of dye, organic solvent used for extraction, ratio of organic phase to aqueous phase during extraction, shaking time and temperature against a reagent blank and the results are graphically presented in (Fig. 5). The absorbance curves show a maximum absorbance at 410 nm. The absorption maximum for BCG in aqueous medium was found to be 410 nm.

Method - M6

The absorption spectrum of the resulting colored species by treating fixed quantity of each SPI separately with F-C reagent was scanned in the wavelength region of 400 - 800 nm with the effects of various parameters such as volume of F-C reagent nature of base and itsconcentration for color development, time for maximum color development, order of addition of reagents and the stability of the colored species formed against a reagent blank and the results are presented graphically in (Fig. 6). The absorbance curves show a maximum absorbance at 770 nm.

Method - M7

To record the spectral characteristics, the absorption spectrum of a solution containing fixed amount of the SPI with the reagent was scanned in the wavelength region of 400 - 800 nm against a reagent blank and the results are presented graphically in (Fig. 7). The wavelength of maximum absorbance was found to be 570 nm with the effect of various parameters such as the effect of reagent concentration, effect of heating time on color development, effect of solvent for final dilution, stability of the colored species where as the blank (omitting the drug) in this method has low or no absorption in this region

Method - M8

In order to ascertain the optimum wavelength of maximum absorption (λmax) specified amount of SPI and DCQC was taken and the color was developed. The absorption spectrum was scanned on a spectrophotometer in the wavelength region of 400 - 800 nm with the effect of various parameters such as the effect of volume of DCQC, solvent for final dilution and stability of the colored species against a reagent blank. The results are graphically presented in (Fig. 8). The wavelength of maximum absorption was found to be 540nm.

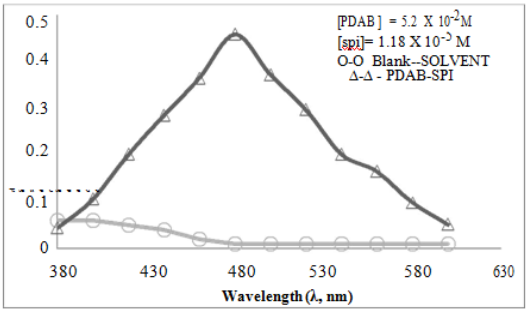


Figure – 1: Absorption spectra of SPI-PDAB (M1)

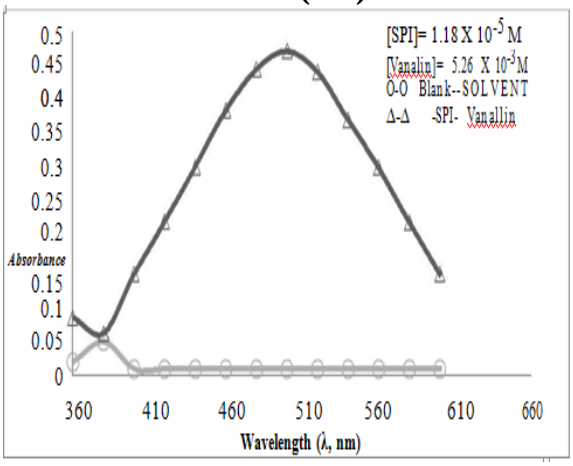


Figure – 2: Absorption Spectra of SPI - Van system M2

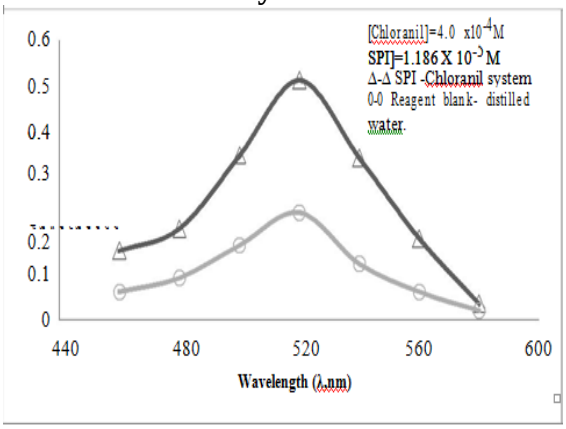


Figure – 3: Absorption Spectra of SPI - Chloranil system M3

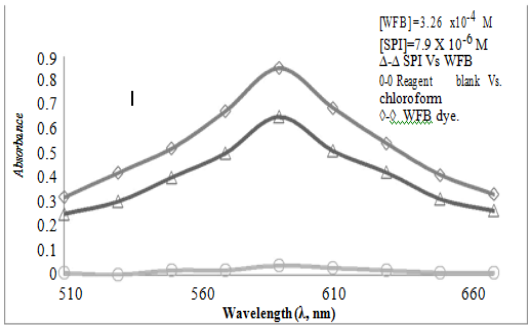


Figure – 4: Absorption spectra of SPI - WFB M4

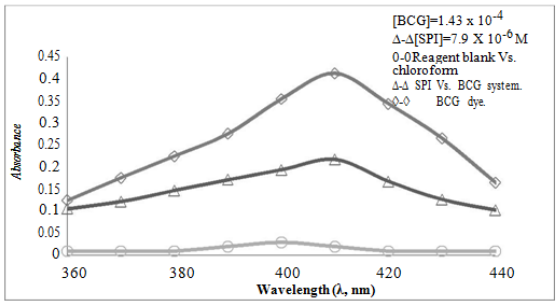


Figure – 5: Absorption spectra of SPI-BCG M5

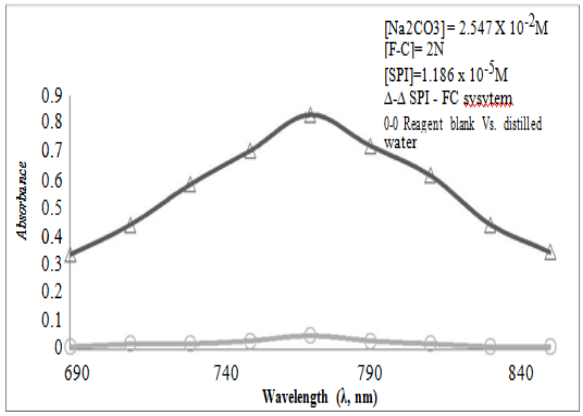


Figure – 6: Absorption spectra of SPI- F.C M6

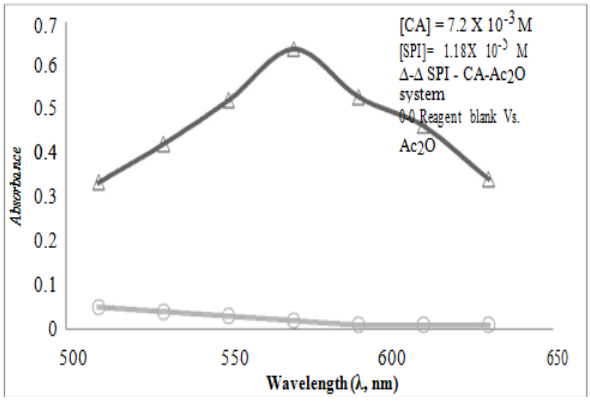


Figure – 7: Absorption spectra of SPI - Citric acid -Ac2O M7

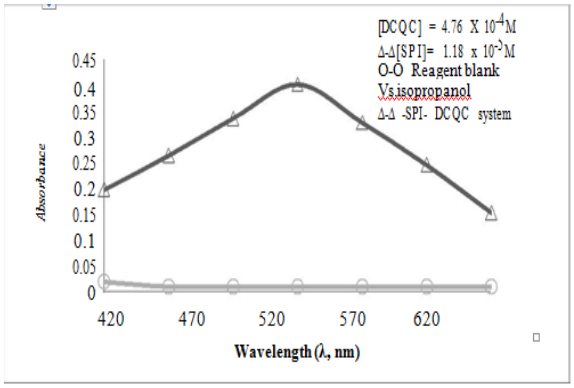


Figure – 8: Absorption spectra of SPI - DCQC system M8

Procedures used for Reference method

Accurately weighed 100 mg of pure or pharmaceutical preparation (tablet was dissolved in acetic acid, filtered through Whatman No. 41 filter paper, the filtrate was made to 100 ml with glacial acetic acid. 10 ml of this solution was made to 50 ml with glacial acetic acid to get a concentration of 200 µg/ ml.

Aliquots of drug solution ranging from 0.5 - 3.0 ml (200 µg/ ml) were taken into a series of 10 ml calibrated tubes. To each of these tubes 2 ml of o-nitrobenzaldehyde (2.64 x 10^{-2}M)followed by 3 ml of concentrated HCl were added. The mixture was shaken and left for 15 min. The volumes were then adjusted to 10 ml with glacial acetic acid. The absorbances were measured before 20 min at 480 nm against reagent blank.

Precision

The precision of each proposed method was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of the drug (two-third the amount of the upper Beer's law limit). The percent relative standard deviation and percent range of error (confidence limits 0.05 and 0.01 levels) were calculated and summarised in Table 1

Accuracy

To determine the accuracy of the proposed methods (M1 – M8) different amounts of bulk samples of each drug (within Beer's law limits) were taken and analysed by the proposed methods and the results (percent error) are given in Table 1.

Table – 1: Optical and regression characteristic, precision and accuracy of the proposed methods for SPI.

Parameter	PDAB	Vanillin	Chloranil	WFB	BCG	FC	CA-AC	DCQC
	M1	M2	M3	M4	M5	M6	M7	M8
λ max(nm)	480	500	520	590	410	770	570	540
beer's law limit µg/ml	5.0 - 50	5.0 - 50	2.5 - 15	2.5-15	5.0-30	2.5 - 10	2.5 -15	2.5 - 15
detection limit µg/ml	0.0541	0.038	0.015	0.045	0.175	0.039	0.713x	0.157
							10-Mar	
Sandle sensitivity	0.059	0.067	0.0194	1.53 x	1.36 x	0.012	0.015	0.025
				10-Feb	10-Jan			
€ max	1.4247 x 104	1.256x 104	4.324x 104	5.4967 x 104	6.1543 x103	7.0226x 104	5.1594x 104	3.389x 104
Regretion equationY =a+Bc Slope (b)	0.016	0.0161	0.06	0.06	0.01	0.09	0.06	0.02
Standard deviation on slope (Sb)	0.99x	1.0 x 10^{-5}	3.088x	0.995 x	0	1.695 x	0	0

	10-May		10-Mar	10-Apr		10-Apr		
Intercept (a) Standard deviation on	0.0082	-0.011	-0.09	0.01	0	-0	-0	0.2
intercept (Sa)	2.89x	2.074x	3.007x 104	0.97x	0.33	1.16 x 10^{-3}	1.48	1.072 x
Standard	10-Apr	10-Apr		10-Mar	10-Apr		10-Apr	10-Apr
deviation on								
estimation (Se)	4.5 x 104	4.98x	3.23x	1.041 x	0.64	9.48 x	1.451x	1.044 x
Correlation coefficient (r)		10-Apr	10-Apr	10-Mar	10-Mar	10-Apr	10-May	10-Mar
Relative standard deviation (%) *	0.9999	0.9999	1	1	1	1	1	1
% Range of error (Confidence limit)	0.76	0.763	0.106	0.21	0.38	0.14	0.18	0.32
0.05 level	0.97	0.877	0.121	0.24	0.43	0.15	0.2	0.41
0.01 level	1.532	1.375	0.191	0.37	0.68	0.23	0.32	0.64
% Error in bulk samples *	0.42	-0.47	0.27	-0.2	-0.4	0.38	0.12	-0.27

Recovery studies

Recovery studies were conducted by analysing each pharmaceutical preparation in the first instance for the active ingredient by the proposed/methods. 10 mg of the drug was added to each one of the previously analysed pharmaceutical preparations and the total amount of the drug was once again determined by the proposed methods (M1 – M8) after bringing the concentration within Beer's law limits. The results are recorded in Table 2.

Analysis of formulations

To find out the stability of the proposed methods for the assay of pharmaceutical formulations, different dosage forms (tablets or capsules) containing selected drugs were analysed by each proposed method and the appropriate reference method (procedures have been given earlier). The results obtained from each of the proposed method were compared statistically by the t and F-tests and were found that these proposed methods not to differ significantly in precision and accuracy from reference method. These results were recorded in Table 2.

Interference studies

The effect of wide range of excipients and other additives usually present in

formulations on the determinations under optimum conditions were investigated. Interference of diverse ions and usually existing excipients and other active ingredients in the determination of each drug by the recommended procedure (M1 – M8) were studied. It was found that the various ingrediants usually present in pharmaceutical preparations do not interfere in the estimation of chosen drugs in the proposed methods.

Formulation a	Labelled amount(mg)	Amount methods b Found by proposed				Reference method	% recovery by proposed methods[c]			
		M1	M2	M3	M4		M1	M2	M3	M4
		PDAB	Vanallin	Chloranil	BCG					
TAB 1	500	500± 0.21	500± 0.49	500± 0.20	500± 0.45	500± 0.32	100±0.23	99.92± 0.58	99.85± 0.25	100.22 ±0.31
		F =2.32	F=1.48	F=2.56	F=1.97					
		t=0.11	t=0.71	t=0.71	t=1.72					
TAB 2	500	500± 0.71	500± 0.71	500± 0.77	500± 0.81	500± 0.76	100.12 ±0.12	99.97± 0.16	100.04 ±0.24	99.88± 0.11
		F=1.14	F=1.14	F=1.02	F=1.13					
		t=0.21	t=1.96	t=1.87	t=.24					
TAB 3	500	500± 0.52	500± 0.47	500± 0.41	500± 0.61	500± 0.49	100.18 ±0.21	99.97± 0.17	100.12 ±.33	100.04 ±0.22
		F=1.12	F=1.08	F=1.42	F=1.54					
		t=1.82	t=2.11	t=1.63	t=1.45					
TAB 4	500	500± 0.39	500± 0.51	500± 0.66	500± 0.65	500± 0.46	100.02 ±0.17	100.14 ±0.24	99.96± .13	99.72± 0.26
		F=1.39	F=1.22	F=2.05	F=1.99					
		t=1.82	t=1.89	t=0.34	t=0.22					

a= Difference batches of tablets from four different pharmaceutical companies.

b = Average ± standard deviation of six determinations, the t- and F- test values refer to comparison of the proposed method with the reference method.
Theoretical values at 95% confidence limit, F= 5.05, t= 2.57.

C = Recovery of 10 mg added to the pre analysed pharmaceutical formulations (average of three determinations).

Table – 2: Assay of SPI in pharmaceutical formulations.

Formulation a	Labelled amount(mg)	Amount found (mg) using proposed methodsb				Found by reference method	% recovery by proposed methods[c]			
		M5	M6	M7	M8		M5	M6	M7	M8
		WFB	F-C	CA-AC2O	DCQC					
TAB 1	500	500± 0.39	500± 0.20	500± 0.20	500± 0.46	500± 0.32	100.17 ±0.18	100.08 ±0.25	99.95± 0.14	99.86± 0.22
		F=1.48	F=2.56	F=2.56	F=2.06					
		t=1.89	t=0.23	t=0.13	t=0.19					
TAB 2	500	500± 0.92	500± 0.66	500± 0.86	500± 0.59	500± 0.76	100±0.23	99.72± 0.26	100.22 ±0.15	99.83± 0.33
		F=1.46	F=1.32	F=1.28	F=1.65					
		t=0.33	t=0.28	t=0.21	t=.20					
TAB 3	500	500± 0.52	500± 0.75	500± 0.59	500± 0.44	500± 0.49	99.78± 0.17	100.08 ±0.19	99.92± 0.31	100.12 ±0.21
		F=1.12	F=2.34	F=1.44	F=1.24					
		t=1.9	t=0.49	t=0.31	t=1.91					

a= Difference batches of tablets from four different pharmaceutical companies.

b = Average ± standard deviation of six determinations, the T- and F-test values refer to comparison of the proposed method with the reference method.

Theoretical values at 95% confidence limit, F= 5.05, t= 2.57.

C = Recovery of 10 mg added to the preanalysed pharmaceutical formulations (average of three determinations).

DISCUSSION

Eight reagents (PDAB, vanillin, chloranil, WFB, BCG, F-C, citric acid - acetic anhydride and DCQC) have been developed for the determination of SPI in eight proposed methods (M1, M2, M3, M4, M5, M6, M7 and M8). The optimum conditions incorporated in the proposed procedures (methods M1 – M8) for the determination of SPI were established with utmost care through control experiments based on the maximum color development and stability of the colored species formed in different reactions.

he validity of the proposed methods for the determination of SPI were established from the precision (calculating percent relative standard deviation, percent range of error at confidence limits with p = 0.05 and 0.01 levels from six determinations) and accuracy (percent error in pure samples, comparison of results obtained with proposed and

reported methods in the case of pharmaceutical preparations and recovery experiments) studies. The sensitivity of each method was ascertained through molar extinction coefficient, Sandell's sensitivity and Beer's law limits. The regression analysis using the method of least squares was made for slope (b), standard deviation (Sb), intercept (a), standard deviation on intercept (Sa), standard error of estimation (Se) and correlation coefficient (r) obtained from different concentrations was shown in Table-1. The selectivity (or specificity) of each proposedmethod was ascertained through interference studies with other active and inactive ingredients usually present in pharmaceutical preparations. The results obtained from each of the proposed methods and reference were compared statistically by the t- and F-test and were found that these proposed methods not to differ significantly in precision and accuracy from reference method (Tables-2). The sensitivity data (λmax, Beer's law limits) of the proposed and reported visible spectrophotometric methods in the determination of individual drugs is furnished in Table 1.

The λmax and εmax values of the colored species formed for SPI in descending order M6> M4> M7> M8> M3> M2> M1> M5 and M2< M1< M8< M3< M7< M4< M5< M6 respectively. The ascending order of precision is M3< M6< M7< M4< M8< M5< M2< M1. The accuracy in these methods is within 1.5%. There is good agreement between the values obtained from the reference and proposed methods in the assay of pharmaceutical formulations (Table-2). The descending order of sensitivities of proposed visible spectrophotometric methods for the drug is as follows. M2< M1 < M8< M3< M7< M4< M5< M6.

CONCLUSION:

Among the various physicochemical methods available for the development of new assay procedures of condensation, charge transfer and ion association complex formation reactions for the spectroscopic determination visible spectrophotometry combines the advantages of low cost and simplicity with the possibility of achieving high sensitivity and relatively with good precision, accuracy and reliability. We found that there is a need for new procedures for the assay of spiramycin (SPI)

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