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SENSITIVE AND SELECTIVE SPECTROPHOTOMETRIC DETERMINATION OF SPIRAMYCIN IN PURE FORM AND IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT: Three simple and sensitive and reproducible spectrophotometric methods were developed for the determination of spiramycin (SPM) in pure form and in pharmaceutical formulations (tablets). The first and second methods, A and B, are based on the formation of charge transfer complex between drug and the chromogenic reagents quinalizarin (method A) and alizarin red S (method B) producing charge transfer complexes in methanolic medium which showed an absorption maximum at 568 and 527 nm using methods A and B, respectively. Beer's law was obeyed in the concentration range of 1.0–10 and 2.0-18 µg mL^{-1} with mean percentages accuracy of 100.39±0.89 and 100.26±0.60 using methods A and B, respectively. The third method C, is based on the reduction of Fe(III) by spiramycin in acid medium and subsequent interaction of Fe(II) with ferricyanide to form Prussian blue, which exhibits an absorption maximum at 760 nm. Beer's law was obeyed in the concentration range of 2.0–12 µg mL^{-1} with mean percentage accuracy of 99.85±0.956. All variables were studied to optimize the reaction conditions for each method. The molar absorptivity, Sandell sensitivity, detection and quantitation limits were calculated. Statistical treatment of the results reflects that the proposed methods are precise, accurate and easily applied for the determination of spiramycin in pure form and in tablets and the results were statistically compared with that reported method.

INTRODUCTION: Spiramycin (SPM) belongs to the class of 16-membered macrolide antibiotics and it is considered to be a medium-spectrum antibiotic with high effectiveness against Gram-positive bacteria. SPM is chemically designated as (6*R*, 7*R*, 9*R*, 10*R*, 11*E*, 13*E*, 16*R*)-10-{[(2*R*,5*S*,6*R*)-5-(dimethylamino)-6-methyltetra-hydro-2*H*-pyran-2-yl]oxy}-5,9,16-trimethyl-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-glu-copyranoside (**Figure 1**). SPM used to treat infections of the oropharynx, respiratory system and genitourinary tract as well as cryptosporidiosis and toxoplasmosis [1]. It is well absorbed after oral administration and distributed in the tissues, especially lungs, liver and kidney [2].

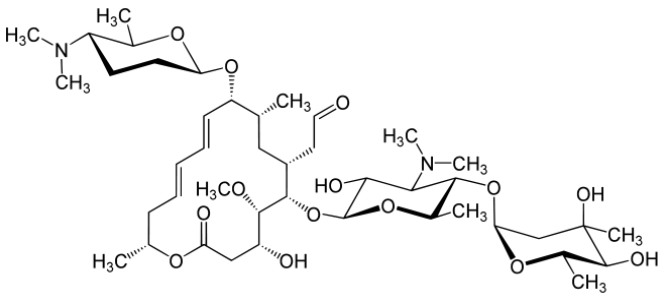


FIGURE 1: CHEMICAL STRUCTURE OF SPIRAMYCIN (SPM)

Several methods have been reported for the determination of SPM in its single pharmaceutical formulations, including titrimetry [3], spectrophotometry [4, 5], TLC [6], LC [7-14], HPLC [15-17], capillary electrophoresis [18], voltammetry [19, 20], immunological assay [21], potentiometry [22] and differential pulse polarographic (DPP) and square wave polarographic (SWP) techniques were developed for the determination of SPM in bulk and tablet forms [23].

None of these techniques is sufficiently sensitive or they are very laborious and require highly sophisticated instrumentation. The reported spectrophotometric methods for determination of SPM in pharmaceutical preparations [4, 5] are not selective, have low sensitivity, take a long time for analysis. To date, no work has been performed to use charge transfer or redox reaction for the determination of SPM.

The scientific novelty of the present work is that the present methods used are very simple, rapid, accurate, selective, sensitive, less expensive and less time consuming than other published LC, TLC and HPLC methods.

The focus of the present study was to develop and validate spectrophotometric methods for the simultaneous determination of SPM in pure form and in pharmaceutical formulations. The proposed methods involves the formation of charge transfer complex between SPM and alizarin derivatives; quinalizarin (method A) and alizarin red S (method B) as chromogenic reagents and redox reaction between SPM and Fe(III) with ferricyanide (method C).

MATERIAL AND METHODS:

Apparatus: All absorption spectra were made using Optima UV-VIS spectrometer (SP-3000 plus) (Tokyo, Japan) and Kontron 930 (UV-Visible) spectrophotometer (German) with a scanning speed of 200 nm/min and a band width of 2.0 nm, both equipped with 10 mm matched quartz cells. The pH values of different buffer solutions were checked using an Hanna pH-meter instrument (pH 211) (Romania).

Materials and Reagents: All chemicals used were of analytical grade and all solvents were of spectroscopic grade.

Samples:

(a) **Pure sample:** Spiramycin (SPM), was kindly supplied as a gift sample by Pharonia Pharmaceuticals (New Borg El-Arab City, Alexandria, Egypt.) and used without further purification. The purity of SPM was found to be 99.95 ± 0.93%.

(b) **Market sample**: Rovamycin® tablets manufactured by (Sanofi aventis, Egypt) were labeled to contain 3.0 M.I.U. SPM per tablet.

Stock standard solutions:

(a) **100 µg mL^{-1} and 1.0 x 10^{-3}M SPM in methanol:** Prepared by simple dissolution of 0.01 and 0.0843 g of the pharmaceutical pure drug, respectively in approximately 10 mL of methanol and further dilution to 100 mL with the same solvent in a volumetric flask.

(b) **100 µg mL^{-1} and 2.0 mg mL^{-1} SPM in methanol- water (10% v/v):** Prepared by dissolving 0.01 and 0.2 g of pure drug, respectively in 10 mL methanol and made up to 100 mL with bidistilled water.

Reagents:

(a) Quinalizarin, 1, 2, 5, 8-tetrahydroxyanthra quinone (Quinz) and alizarin red S, 3, 4-dihydroxy-9, 10-dioxo-2-anthracene sulfonic acid (ARS) were Aldrich products and used without further purification. A stock solution 1.0 x 10^{-3} M was prepared by dissolving the appropriate weight of the reagent in approximately 25 mL of dimethyl sulfoxide (DMSO). After obtaining a solid-free solution, it was transferred to a 100 mL volumetric flask and the volume was completed to the mark with DMSO. This solution was stable for one week, at least.

(b) Anhydrous $FeCl_3$ (Merck) and $K_3[Fe(CN)_6]$ (BDH Lab. Chemicals, Poole, England); 0.2% (w/v) were prepared in bidistilled water. Hydrochloric acid (1.0 M) was prepared, (Sp. Gr. 1.18) [24].

Construction of calibration curves

Methods A and B: To a set of 10 mL volumetric flasks, appropriate aliquots of the standard working solution were transferred, to obtain concentrations in the range (0.1-1.8 µg mL^{-1}) of SPM. To each flask 1.0 and 2.0 mL of (1.0 x 10^{-3} M) Quinz and ARS solutions, respectively were added. Afterwards, the obtained mixture was shaken in order to promote the reaction and the volume was completed to the mark with methanol. The absorbance of the resulting solutions were measured at 568 and 527 nm using methods A and B, respectively against a reagent blank prepared simultaneously. The calibration graph was constructed by plotting the absorbance *versus* the final concentration of the drug. The corresponding regression equation was derived.

Method C: Into a series of 10 mL calibrated flasks, different aliquots (0.2-1.2 mL) of 100 µg mL^{-1} SPM standard solution were transferred using a micro pipette and the total volume was adjusted to 3.0 mL by adding bidistilled water. Then, 1.0 mL of $FeCl_3$ (0.2 %) and 1.0 mL of $K_3[Fe(CN)_6]$ (0.2 %) were added to each flask, mixed well and let to stand for 10 min. Finally, 1.0 mL of 1.0 M HCl was added to each flask and diluted to mark with bidistilled water and mixed well. The absorbance of the resulting solution was measured at 760 nm against reagent blank prepared similarly. Calibration graph was prepared by plotting absorbance versus concentration of drug and the concentration of the unknown was read from the calibration graph or computed from the regression equation derived from the Beer's law data.

Applications to pharmaceutical formulations: The contents of ten tablets were removed and finely powdered using an agate mortar. The combined contents were mixed and weighed accurately. A portion of the powder equivalent to 50 mg of the drug was accurately weighed and exactly 25 mL of methanol was added, sonicated for about 20 min, left for a time in a refrigerator to allow any insoluble matter to settle down and then filtered into a 100 mL volumetric flask. The solution was then completed to volume with methanol for methods A and B or with bidistilled water for method C. Working standard solutions were prepared from suitable dilution of the standard stock solution and the procedure was completed as described for preparing the calibration graph.

The nominal contents of the tablets were determined either from the calibration graphs or using the corresponding regression equations.

Stoichiometric relationship: The stoichiometric ratios of the reaction products formed between SPM and reagents were determined by applying the continuous variation method attributable to Job [25] and modified by Vosburgh and Coober [26], at the optimum wavelengths of maximum absorbance for each method. Job's method of continuous variation was employed; a 1.0×10^{-3} M standard solution of SPM and 1.0×10^{-3} M solution of reagents were used. A series of solution were prepared in which the total volume of drug and reagent was kept at 2.0 mL. The reagents were mixed in various proportions and diluted to volume in a 10 mL calibrated flask with the appropriate solvent following the above mentioned procedures

RESULTS AND DISCUSSION:

Absorption Spectra:

Methods A and B: The study and development of the methods A and B for the determination of SPM in bulk powder and pharmaceutical formulations, exploring its charge transfer reaction with alizarin derivatives (quinalizarin or alizarin red S) in methanol medium was performed through two steps:

(i) Optimization of the experimental conditions in order to achieve both maximum sensitivity and selectivity. This step comprised the evaluation of the effect of the solvent nature, investigation of the influence of the reagent concentration and evaluation of the time required to complete the reaction and;

(ii) Study and characterization of the reaction, which was carried out by the evaluation of the reaction stoichiometry (Job's continuous variation method) and the verification of the proposed reaction mechanism.

At optimum conditions, the radical anion (absorbing species) was formed in the medium immediately after mixing of the reagents and showed maximum absorption at 568 and 527 nm using methods A and B, respectively in methanol medium (**Figure 2**).

Thus, these wavelengths were chosen for all further measurements in order to obtain highest sensitivity for the proposed methods. It is important to point out that Quinz and ARS alone, in methanol medium, exhibits maximum absorption at, 491 and 422 nm, respectively. The high difference between maxima of the reagent and the product absorption bands ~67 and 105 nm using methods A and B, respectively, allowed the measurement of the products with only a small contribution of both reagents that was added in excess in the medium.

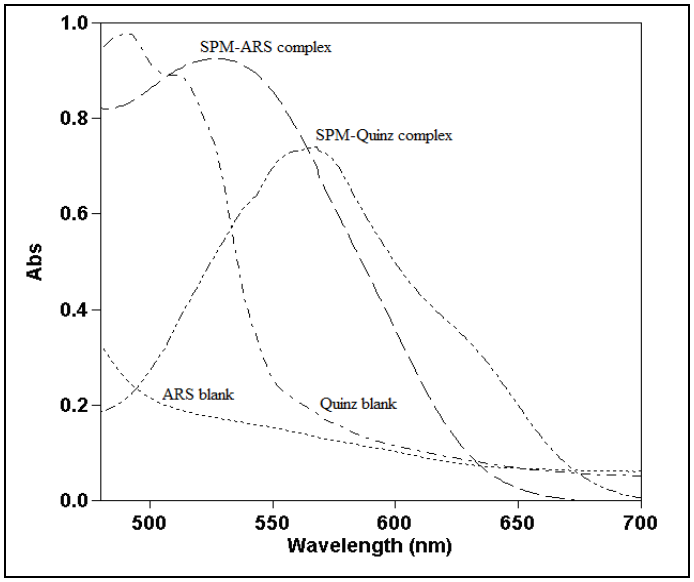


FIGURE 2: ABSORPTION SPECTRA OF CHARGE TRANSFER COMPLEXES OF 10 AND 15 µg mL^{-1} SPM WITH (1.0x10^{-3}M) QUINZ AND ARS, RESPECTIVELY IN METHANOL SOLVENT OBTAINED AGAINST REAGENT BLANK SOLUTION PREPARED IN THE SAME SOLVENT

Method C: SPM is an amine that reduces Fe(III) to Fe(II), the latter reacting with ferricyanide to form intense greenish-blue [27] (Prussian blue, PB) colored chromogen having an absorption maximum at 760 nm. Neither Fe(III) nor ferricyanide solution absorbs at this wavelength. Hence, the use of measured volumes of the reagent solutions and measurement against a corresponding reagent blank give a linear calibration graph for the drug. The formation of PB complex is a classic qualitative test to detect Fe(II) using hexacyanoferrate(III) as reagent [24]. The first step is the oxidation of Fe(II):

$$Fe^{2+} + [Fe(CN)_6]^{3-} \rightarrow Fe^{3+} + [Fe(CN)_6]^{4-}$$

The second step is the formation of hexa cyanoferrate(II) complex (PB):

$$4Fe^{3+} + 3[Fe(CN)_6]^{4-} \rightarrow Fe_4[Fe(CN)_6]_3$$

The complex formed is highly (Ksp = 3.0 × 10^{-41}) [28]. Employing an excess of the complexing reagents, a deep blue soluble compound is formed when Fe (III) is reduced to Fe(II) by products obtained from acidic hydrolysis of the mentioned drug.

Optimization of the Experimental conditions:

Methods A and B:

The effect of the Solvent nature: The solvent plays an important role in some charge transfer reactions, since it must be able to facilitate the total charge transfer and then allow the complex dissociation and stabilization of the radical anion formed, which is the absorbing species. According to the literature, solvents with high dielectric constant are more effective to execute this task [29]. Taking this fact into account, water would be an excellent solvent for the procedure.

However, the poor solubility of the reagents in water did not allow its use in the present case. So, the reaction was tested in ethanol, methanol, acetone, DMSO and acetonitrile medium. Although the highest dielectric constant of DMSO and acetonitrile, best sensitivity was achieved with methanol, probably because of the capacity of this solvent to form stable hydrogen bonds with the radical anion. Then, methanol was chosen for further experiments (**Figure 3**).

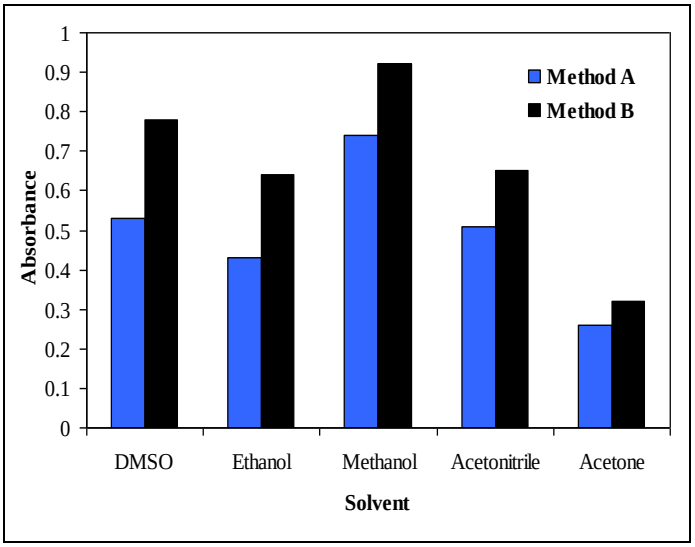


FIGURE 3: EFFECT OF DIFFERENT SOLVENTS ON THE CHARGE TRANSFER COMPLEX OF QUINZ-SPM SOLUTION OBTAINED AGAINST (1.0X10^{-3} M) QUINZ

SOLUTION PREPARED IN EACH SOLVENT. SPM concentration = 20 μg mL^{-1}.

Effect of the Reagent concentration: The reagent concentration in solution is an important parameter to be studied, since the maximum conversion of the analyte into absorbing species depends on the amount of the reagent available in the solution for the reaction and equilibrium involved. In order to achieve this objective, an experiment was performed when various volumes of reagents solutions (1.0 x 10^{-3}M) in the range of 0.5-5.0 mL were added to a fixed drug concentration (10 and 15 μg mL^{-1}) using Quinz and ARS reagents, respectively (**Figure 4**). The results shows that 1.0 and 2.0 mL of (1.0 x 10^{-3}M) Quinz and ARS reagent solutions, respectively were enough to give the highest and constant absorbance values.

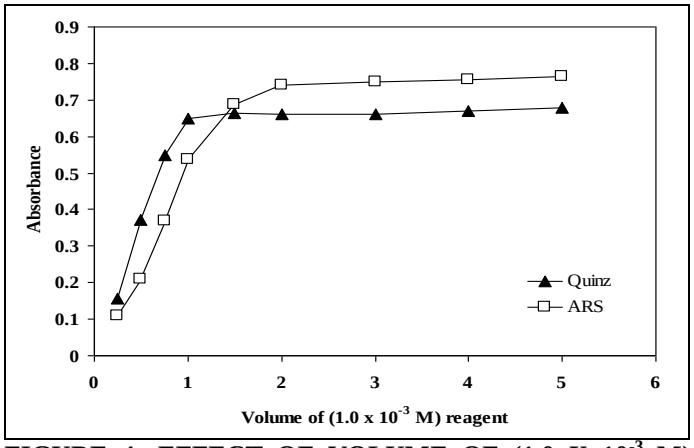


FIGURE 4: EFFECT OF VOLUME OF (1.0 X 10^{-3} M) QUINZ OR ARS REAGENTS ON THE ABSORBANCE OF SPM- REAGENT CHARGE TRANSFER COMPLEXES AT THE OPTIMUM WAVELENGTHS.

Effect of the Reaction time and Temperature: The optimum reaction time was determined by continuous monitoring of the absorbance and color development at optimum wavelengths and at laboratory ambient temperature (25±2°C). Complete color development was attained after 3.0 min with both reagents. On raising the temperature, the absorbance of the charge transfer complexes were decrease with a hypochromic shift, until decayed at 60 °C.

Sequence of Additions: The most favorable sequence of addition is "drug-reagent-solvent" for complete color development, highest absorbance and stability at the recommended wavelength. Other sequences needed longer time in addition to lower stability. The complexes with this sequence remain stable for at least 24 h.

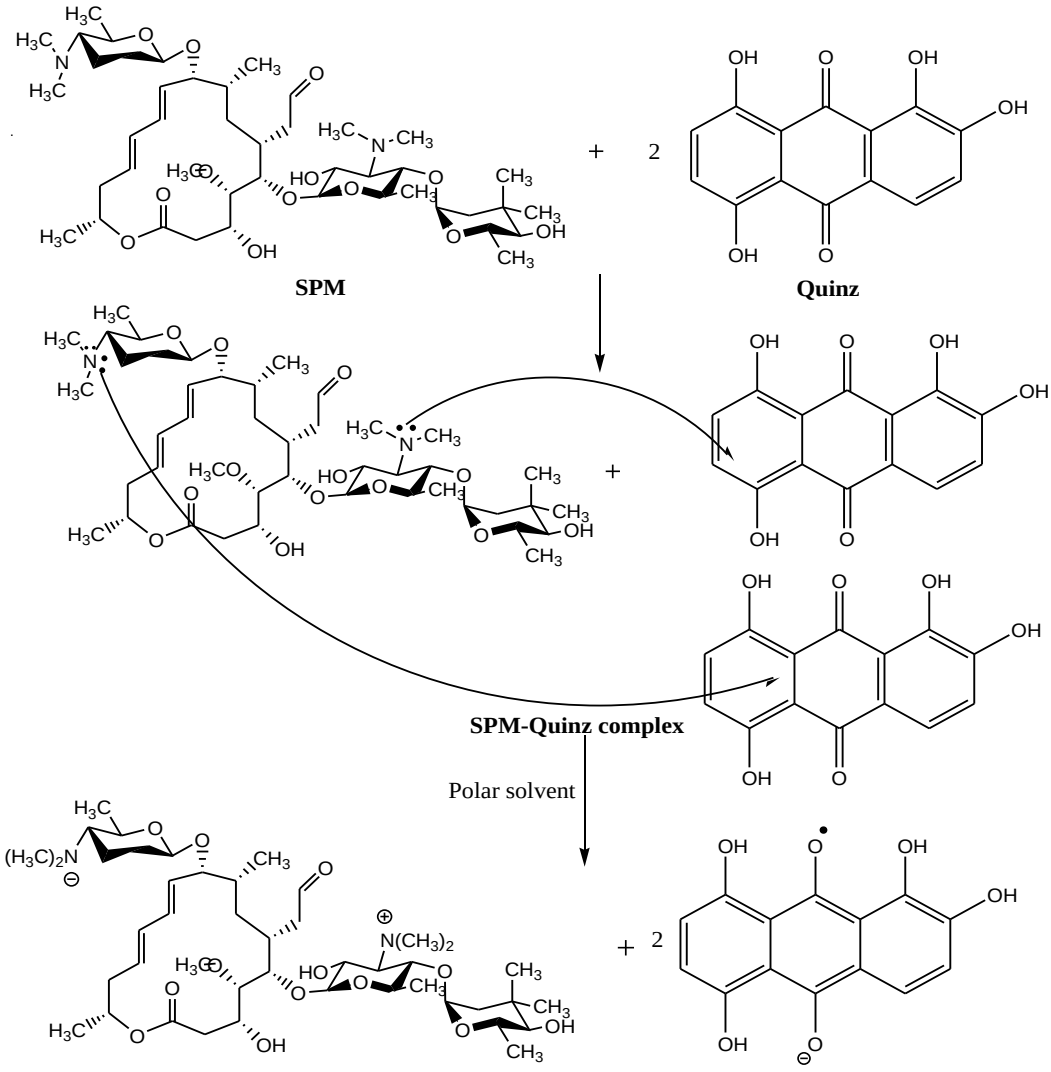
Mechanism of the Reaction: Solutions of Quinz and ARS reagents in methanol exhibits an absorption bands with a well-defined maximum at 491 and 422 nm for Quinz and ARS, respectively, while the drug solution in methanol showed no absorption in the 400-700 nm range. The addition of drug to reagent solution in methanol caused an immediate change in the absorption spectrum with the appearance of a new characteristic band with maximum absorption at optimum wavelengths recorded in **Table 1**.

TABLE 1: ANALYTICAL PARAMETERS FOR THE DETERMINATION OF SPM BY THE PROPOSED METHODS

Parameters	A	B	C
Wavelengths λ_{max} (nm)	549	555	760
Beer's law limits (μg mL^{-1})	1.0-10	2.0-18	2.0-12
Molar absorpitivity ε, (L mol^{-1} cm^{-1}) x 10^4	6.6036	3.7666	5.610
Sandell sensitivity, (μg cm^{-2})	12.77	22.38	13.33
Regression equation [a]			
Intercept (a)	0.0083	0.0072	-0.0008
Slope (b)	0.0744	0.0434	0.0647
Correlation coefficient (*r*)	0.9996	0.9998	0.9997
Mean recovery % ± SD [b]	100.39±0.89	100.26±0.60	99.85± 0.956
Relative standard deviation (RSD%	0.887	0.598	0.957
Relative error (RE%)			1.005
Variance	0.792	0.36	0.914
Detection limits (μg mL^{-1})	0.062	0.287	0.399
Quantification limits (μg mL^{-1})	0.20	0.957	1.329
Calculated t-value (2.257) [c]		0.46	0.82
Calculated F-value (5.05) [c]		1.35	1.39

[a] $A=a + bC$, where C is the concentration in (μg mL^{-1}), A is the absorbance, a is the intercept and b is the slope. [b] Average of six determinations. [c] Theoretical values for five degree of freedom and 95 % confidence level at p = 0.05**.**

According to Gouda and Kassem, 2012 [30] molecular charge-transfer complexes are formed in non-polar solvents while radical anion species are predominant in polar solvents. Also, it is believed that the addition of basic compounds that contains a lone pair of electrons, such as SPM, results in the formation of charge-transfer complexes of n-π type. This kind of complexes can be considered an intermediate molecular-association compound that forms a corresponding radical anion in polar solvents. In this case, radical anions results from the total transfer of charge (**Scheme 1**).



SCHEME 2: POSSIBLE MECHANISM OF RADICAL ANION FORMATION FROM QUINZ AND SPM REACTION

Method C:

Effect of Iron(III) and Ferricyanide concentrations: When a study on the effect of iron (III) chloride concentration on the color development was performed, it was observed that the absorbance increased with increase in the volume of 0.2% iron (III) and ferricyanide solutions and reached maximum when 1.0 mL of each of 0.2% Fe (III) and ferricyanide reagent solutions was added to 10 µg mL^{-1} of SPM in a total volume of 10 mL was used to ensure adequate reagent concentrations for higher drug concentrations (**Figure 5**).

These results indicate that a maximum absorbance is obtained when the final Iron (III) chloride or ferricyanide concentration is 0.02%. Larger volumes of iron (III) chloride or ferricyanide up to 3.0 mL had no effect on the sensitivity of the reaction.

The results of this study reveal that the concentrations of iron(III) and ferricyanide reagents are not critical.

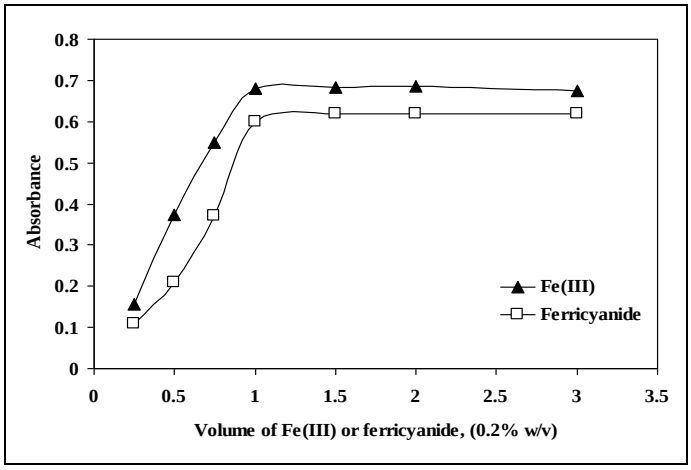


FIGURE 5: EFFECT OF IRON(III) CHLORIDE AND FERRICYANIDE CONCENTRATION (0.2% w/v) WITH 10 µg mL^{-1} DRUG AND 1.0 mL of 1.0 M HCl

Effect of nature of acid and its concentration: The reaction product PB was found to flocculate within 20-30 min of color development. To delay the flocculation, addition of acid after full color development and before diluting to the mark was found necessary.

Hydrochloric acid was found to give more stable color and reproducible results compared to sulphuric acid. A 1.0 mL volume of 1.0 M hydrochloric acid in a total volume of 10 mL was found adequate for the purpose.

Effect of Reaction temperature, Time and Stability on Colored species: The effect of temperature on colored product was studied at different temperatures. It was found that the colored product was stable in the temperature range 0-50 °C. At higher temperatures, the blue and bluish green clear solution precipitates out and blanks solution develops a light color and also decrease in the absorbance was observed.

The reaction is slow at 25±5°C, but the absorbance increases with time and reaches a maximum in 10 min. However, the color product remained stable for at least 6.0 h at room temperature.

Effect of 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 Fe(III) followed by hydrochloric acid after full development of color gave maximum absorbance and stability, and hence the same order of addition was followed throughout the investigation.

Stoichiometric ratio: Under the optimum conditions, the stoichiometry of the charge transfer complexes formed between SPM and Quinz or ARS reagents, respectively were investigated by applying the Job's method of continuous variation [25] and modified by Vosburgh and Coober [26] at the wavelengths of maximum absorbance, keeping the sum of the molar concentrations of SPM and reagents fixed. The results obtained showed that the stoichiometric ratio of the charge transfer complexes is (1:2) (drug: reagent) (**Figure 6**).

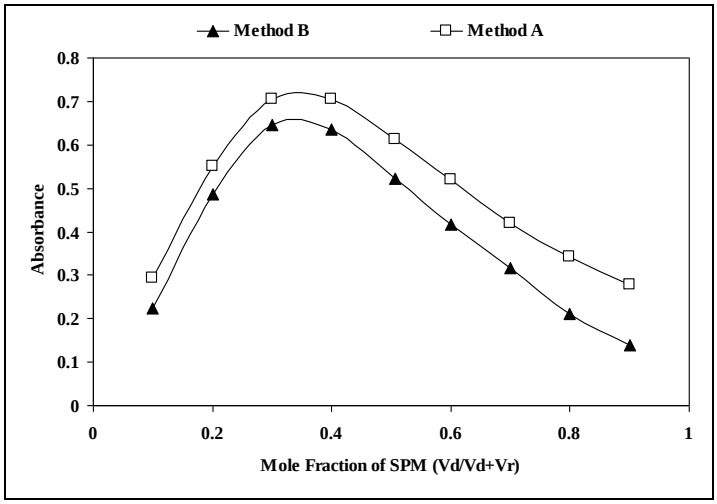


FIGURE 6: APPLICATION OF JOB'S METHOD TO THE REACTION BETWEEN SPM AND (1.0 x 10^{-3} M) QUINZ OR ARS REAGENTS. Absorbance measurements were carried out at optimum wavelength (nm).

Validation of the proposed methods: The validity of the proposed methods was tested regarding linearity, specificity, accuracy, repeatability and precision according to International Conference on Harmonization (ICH) [31] guidelines.

Linearity, Detection, and Quantification Limits: Under the experimental conditions of the proposed methods, linear regression equations were obtained. The regression plots showed that there was a linear dependence of the analytical response in the five methods to the concentration of the drug over the ranges cited in Table 1. Linear regression analysis of the data gave the following equations. A= 0.0744C + 0.0083 (r= 0.9996) method A; A= 0.0434C + 0.0072 (r= 0.9998) method B and A= - 0.0008 + 0.0647C, (r= 0.9997) method C. Where A is the absorbance, C is the concentration of the drug (µg mL^{-1}), and r is the correlation coefficient. The detection limit (LOD) is defined as the minimum level at which the analyte can be reliably detected for the three drugs was calculated using the following equation [31, 32]:

$$LOD = 3s / b$$

Where, *s* is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and *b* is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limits were found to be 0.062, 0.287 and 0.399 µg mL^{-1} using methods A, B and C, respectively. The limit of quantization, LOQ, is defined as the lowest concentration that can be measured with acceptable accuracy and precision [31, 32]:

$$LOQ = 10 s / b$$

According to this equation, the limit of quantization was found to be 0.20, 0.957 and 1.329 µg mL^{-1} using methods A, B and C, respectively.

Accuracy and precision: Percentage relative standard deviation (RSD%) as precision and percentage relative error (Er %) as accuracy of the proposed methods were calculated. Precision was carried out by analyzing six samples of the studied drug at four different concentration levels. The relative standard deviation (RSD) values were less than 2.0% in all cases, indicating good repeatability of the suggested methods. The percentage relative error calculated using the following equation:

$$Er\ \% = [(founded - added) / added] \times 100$$

The inter-day and intra-day precision and accuracy results show that the proposed methods have good repeatability and reproducibility (**Table 2**).

TABLE 2: INTRA-DAY AND INTER-DAY ACCURACY AND PRECISION DATA FOR THE PROPOSED METHODS ON PURE SAMPLE OF THE INVESTIGATED DRUGS

Method	Intra-day					Inter-day			
	Taken (µg mL^{-1})	Recovery %	Precision RSD %[a]	Accuracy Er %	Confidence limit	Recovery %	Precision RSD% [a]	Accuracy Er %	Confidence limit
A	2.0	100.05	0.62	0.05	2.001 ±0.013	100.30	0.58	0.30	2.006 ±0.012
	4.0	99.55	0.51	-0.45	3.982±0.021	99.60	0.38	-0.40	3.984 ±0.016
	6.0	99.65	0.45	-0.35	5.979±0.028	99.70	0.32	-0.30	5.982 ±0.020
	8.0	99.90	0.35	-0.10	7.992 ±0.029	99.25	0.27	-0.75	7.940 ±0.023
B	4	99.70	0.69	-0.30	3.988±0.029	99.40	0.59	-0.60	3.976±0.025
	8	100.40	0.44	0.40	8.032±0.091	99.60	0.55	-0.40	7.968±0.046
	12	100.25	0.39	0.25	12.03±0.049	100.60	0.52	0.60	12.072±0.066
	16	99.15	0.29	-0.85	15.864±0.048	100.20	0.43	0.20	16.032±0.072
C	3	99.80	0.58	-0.20	2.994±0.018	99.60	0.67	-0.40	2.988±0.021
	6	100.10	0.46	0.10	6.006±0.029	99.40	0.50	-0.60	5.964±0.031
	9	99.45	0.32	-0.55	8.951±0.030	100.25	0.46	0.25	9.023±0.044
	12	99.90	0.26	-0.10	11.988±0.033	100.10	0.31	0.10	12.012±0.039

[a] Mean of six determination; RSD%, percentage relative standard deviation; Er%, percentage relative error.

Recovery studies: To confirm the accuracy of the method, recovery studies were performed by using the point standard addition method [33]. This depends upon the addition of a known quantity of the standard drug to a fixed amount of the corresponding pharmaceutical sample SPM and then analyzing the resulting solution by the proposed methods.

The difference in absorbance of standard and sample plus standard was used to calculate the concentration of sample after each addition. Results indicate good recoveries for SPM prove the lack of interference due to common excipients and, hence, accuracy of the proposed methods (**Table 3**).

Interference studies: The selectivity of the proposed spectrophotometric methods was investigated by observing any interference encountered from some common excipients of the pharmaceutical formulations such as starch, lactose, sucrose, glucose, gum acacia, and magnesium stearate. It was shown that these excipients did not interfere with the proposed methods. So, the proposed methods are able to determine the analyte SPM in the presence of common excipients.

TABLE 3: APPLICATION OF THE STANDARD ADDITION TECHNIQUE FOR THE DETERMINATION OF SPM IN PHARMACEUTICAL PREPARATIONS (ROVAMYCIN® TABLETS) USING THE PROPOSED METHODS

Parameters	A			B			C		
	Taken (µg mL^{-1})	Added (µg mL^{-1})	Recovery [a] (%)	Taken (µg mL^{-1})	Added (µg mL^{-1})	Recovery [a] (%)	Taken (µg mL^{-1})	Added (µg mL^{-1})	Recovery [a] (%)
	1.0	-	100.20	2.0	-	99.80	2.0	-	99.30
		1.0	100.10		4.0	99.00		2.0	100.40
		3.0	99.55		8.0	99.60		4.0	99.50
		5.0	98.90		12	100.20		6.0	100.20
		7.0	99.20		14	100.50		8.0	101.40
		9.0	99.80		16	99.30		10	99.60
Mean ± SD			99.63±0.510			99.73±0.557			100.07±0.779
V			0.260			0.311			0.607
R.S.D			0.512			0.559			0.780
SE			0.208			0.228			0.318

[a] The average of six determinations. [b] RSD, relative standard deviation; SE, standard error; V, variance; SD, standard deviation.

Robustness of the proposed methods was assessed by evaluating the influence of small variation of experimental variables, i.e., concentrations of reagents and reaction time, on the analytical performance of the methods. In these experiments, one experimental parameter was changed while the other parameters were kept unchanged, and the recovery percentage was calculated each time. The small variations in any of the variables did not significantly affect the results. High recovery values and RSD% did not exceed 2.0 % were obtained. This indicated the reliability of the proposed methods during its routine application for the analysis of SPM.

Analysis of Pharmaceutical Formulations: The proposed methods were successfully applied to the determination of SPM in its pharmaceutical formulations (tablets) (**Table 4).**

The methods were tested for linearity, specificity, accuracy, repeatability, and precision according to ICH recommendations. The results of the proposed methods were compared statistically, by applying the *t*- and *F*-tests, with the results obtained by the reference method [20]. Statistical analysis of the results, using Student's *t*-test and the variance ratio *F*-test at 95% confidence level revealed no significant difference between the performance of the proposed and reference methods regarding the accuracy and precision, respectively (Table 4) [32].

It is evident from these results that the proposed methods are applicable to the analysis of SPM in its bulk form and pharmaceutical formulations (tablets) with comparable analytical performance. The critical recommendations of some of these methods might be based on their relative sensitivities (depending upon the amount of specimen available for analysis) and experimental conditions (reaction time, reagent volume, etc.).

TABLE 4: APPLICATION OF THE PROPOSED METHODS TO THE DETERMINATION OF SPM IN DOSAGE FORMS (ROVAMYCIN® TABLETS)

Samples	References method [20]	Proposed methods		
		A	B	C
X ± SD [a]	99.50 ± 0.80	99.63±0.51	99.73±0.557	100.07±0.779
t-value [b] *(2.571)*		0.25	0.44	1.01
F-value [b] (5.05)		2.46	2.06	1.05

[a] Average of six determinations. [b] Theoretical values for five degree of freedom and 95 % confidence level at p = 0.05.

CONCLUSION: The proposed methods in the present work proved to be an excellent alternative for SPM determination in pharmaceutical formulations. It presented adequate sensitivity and selectivity, allowing the determination of the analyte at levels under those found in the samples. Also, the developed methods presented some advantages such as the use of low cost instrumentation and low operational cost. In the practical point of view, the methods required minimum sample treatment, which allowed us to achieve a high analytical productivity. These characteristics make the method very suitable for routine analysis in quality control laboratories.

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