above results demonstrated the antitumour effect of ESE against DAL is Swiss albino mice. A significant enhancement of MST and peritoneal cells counts were observed (Tables 1 and 2).

To evaluate whether ESE treatment indirectly inhibited tumour cell growth, the effect of ESE treatment was examined on the peritoneal exudate cells of normal mice. Normally each mouse contains about $5x10^6$ intraperitoneal cells, 50% of which are macrophages. ESE treatment was found to enhance peritoneal cell counts. These results demonstrated the indirect effect of ESE in DAL cells, probably medicated through enhancement and activation of macrophages or through some cytokine product inside the peritoneal cavity produced by ESE treatment.

Analysis of the haematological parameters showed a minimum toxic effect in mice which were cured by ESE treatment. Fourteen days after transplantaion, ESE treated groups were able to reverse the changes in the haematological parameters consequent to tumour inoculation.

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New Spectrophotometric Methods for the Determination of Roxithromycin

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Two simple spectrophotometric methods (A and B) have been developed for the determination of roxithromycin in pure and its pharmaceutical formulations. Method A is based on the formation of a blood red coloured complex with ferric chloride and 1,10-phenanthroline with absorption maximum at 520 nm. In method B, roxithromycin forms blue coloured complex with Folin-Ciocalteu (FC) reagent in the presence of sodium carbonate exhibiting maximum absorption at 760 nm. The chromogens obey Beer's law in the concentration ranges of 2.5 to 40 µg/ml and 2.5 to 12.5 µg/ml for method A and B, respectively.

Roxithromycin is a broad spectrum semisynthetic macrolide antibiotic and chemically it is erythromycin-9-[O-(2-methoxyethoxy) methyl]oxime[1,2]. Very few analytical methods have been reported for the determination of roxithromycin which include HPLC[3-6] and visible spectrophotometric[7] methods. The authors have developed two simple sensitive and reproducible spectrophotometric methods (A and B) for the determination of roxithromycin.

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In method A, roxithromycin reduces $FeCl_3$ to $FeCl_2$ and Fe^{+2} ions which reacts with 1,10-phenanthroline to form a blood red coloured complex with absorption maximum at 520 nm, whereas in method B it reduces the Folin-Cioalteu (FC) reagent in presence of sodium carbonate to form a blue coloured chromogen exhibiting maximum absorption at 760 nm. The blue coloured complex formed may be due to the reduction of FC reagent and the formation of molybdenum blue or tungstan blue.

All the chemicals used were of analytical grade. Solutions of ferric chloride (Loba Chemie) (0.0033 M), o-phosphoric acid (Qualigens) (0.2 M), 1,10-phenanthroline (Loba Chemie) (0.1 M), FC reagent (Loba Chemie) (1 N) and sodium carbonate (BDH) (10%) were prepared in distilled water. The commercially available tablets of roxithromycin [Roxisara (Sarabhai), Odirox (Protec), Roxitas (Intas) and Rekthro (Rekvina)] were procured from local market. Spectral and absorbance measurements were made on a Systronics UV-Vis spectrophotometer model 117 with 10 mm matched quartz cells.

About 100 mg of roxithromycin (pure or from formulation) was accurately weighed and dissolved in 50 ml of distilled water. To that, 0.2 ml of concentrated HCl was added and the total volume was made up to 100 ml with distilled water. This stock solution was further diluted to get a working standard solution of 100 µg/ml for method A and 250 µg/ml for method B.

In the method A, aliquots of working standard solution of roxithromycin, ranging form 0.25 to 4.0 ml (100 µg/ml) were transferred into a series of 10 ml test tubes. To that, 1.5 ml of $FeCl_3$ (0.0033 M) and 1.5 ml of 1,10-phenanthroline (0.1 M) solutions were successively added. Then the tubes were heated on water bath for 15 min, cooled to room temperature. This reacted solution was then transferred in to a series of 10 ml volumetric flasks and 1 ml of o-phosphoric acid (0.2 M) was added to all the flasks. The final volume was made up to 10 ml with distilled water. The absorbance of the blood red coloured species formed was measured at 520 nm against the reagent blank and the amount of roxithromycin present in the sample solution was computed from its calibration curve. The absorbance of reaction product at 520 nm remains stable for 4 h after final dilution.

In method B, to a series of 10 ml graduated test tubes, aliquots of working standard solutions of roxithromycin, ranging from 0.5 to 6.0 ml (250 µg/ml), were transferred and the volume was made up to 5 ml with distilled water. To that, 1.5 ml of Na_2CO_3 (10%) solution and 1 ml of FC reagent (1N) were added and mixed well. The total volume of each tube was made up to 10 ml with distilled water. The

TABLE 1: OPTICAL CHARACTERISTICS AND PRECISION.

Parameters	Method A	Method B
Beer's law limit (µg/ml)	2.5-40	2.5-12.5
Sandell's sensitivity		
($µg/cm^2$/0.001 absorbance unit)	0.025	0.0236
Molar Extinction Coefficient ($L.mole^{-1}.cm^{-1}$)	$2.87x10^4$	$3.55x10^3$
%Relative Standard Deviation**	0.599	0.600
%Range of Error		
0.05 confidence limits	±0.579	±0.502
0.01 confidence limits	±0.856	±0.742
Correlation Coefficient	0.9998	0.9999
Regression equation (Y*)		
Slope (a)	$3.4x10^{-2}$	$4.24x10^{-4}$
Intercept (b)	$-0.0425x10^{-2}$	$0.08x10^{-2}$

Y*=b+aC, where "C" is concentration in µg/ml and Y is absorbance unit. **Calculated from eight replicated samples.

absorbance of blue coloured chromogen formed was measured at 760 nm against the reagent blank and this chromogen remains stable for 6 h. The amount of roxithromycin present in the sample solution was computed from its calibration curve.

The optical characteristics such as Beer's law limits, Sandell's sensitivity, molar extinction coefficient, percent relative standard deviation, (calculated from the eight measurements containing 3/4th of the amount of the upper Beer's law limits of roxithromycin) and percent range of error (0.05 to 0.01 confidence limits) were calculated for both the methods and the results are summarized in Table 1.

The value obtained for the determination of roxithromycin in different brands of tablets by the proposed and the reported methods are compared in Table 2. To evaluate the validity and reproducibility of the methods, known amounts of pure drug were added to the previously analysed pharmaceutical preparations and the mixtures were analysed by the proposed methods. The percent recoveries are given in Table 2.

These studies revealed that the common exciepients and other additives such as parabens, lactose, sucrose, starch, sodium benzoate, sodium phosphate, calcium gluconate, gelatin, talc and magnesium stearate, that are usually present in the dosage forms did not interfere in the proposed methods.

The blood red coloured complex formed in method A may be due to the fact that each of the two nitrogen atoms in 1,10-phenanthroline has an unshared pair of electrons that can be shared with Fe (II) ion [formed by the reaction of roxithromycin with Fe (III)]. Three such molecules of 1,10-phenanthroline attach themselves to the metallic ion (ferrion complex) as presented in fig. 1. FC reagent is the mixture of phosphoric acid, sodium molybdate and sodium tungstate. It is also called as phosphomolybdotungstic acid. In method B, the colour formation by FC reagent with roxithromycin may be explained in the following manner based on the analogy with the reports of earlier workers[8]. The mixed acids in the FC reagent preparation are the final chromogen and involve the following chemical species; $3H_2O.\ P_2O_5.\ 13WO_3.\ 5MoO_3\ 10H_2O$ and $3H_2O.\ P_2O_5.\ 14WO_3.\ 4MoO_3\ 10H_2O$.

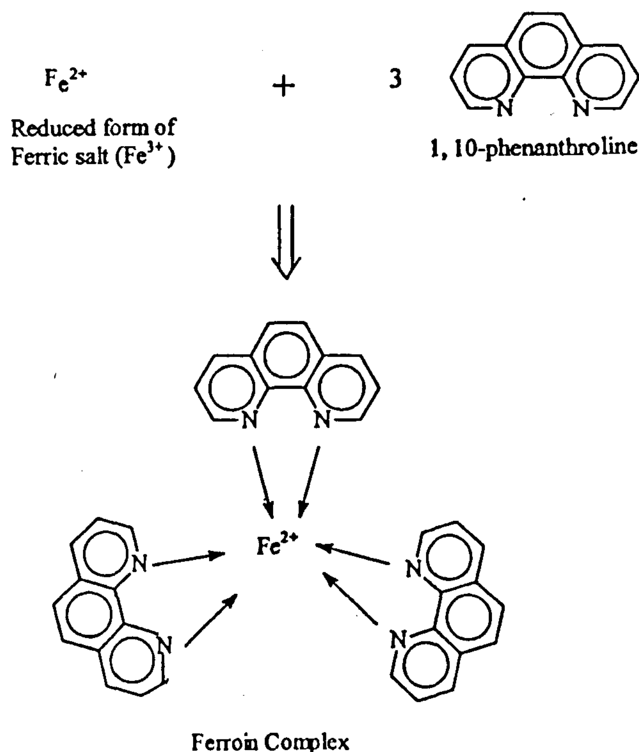

Fig. 1: Reaction complex of 1, 10-phenanthroline with Fe^{2+}.

TABLE 2: ESTIMATION OF ROXITHROMYCIN IN PHARMCEUTICAL FORMULATIONS.

Sample	Labelled Amount (mg)	Amount obtained*(mg)			% Recovery of the Proposed Method**	
		Proposed method		Reported method[7]	Method A	Method B
		Method A	Method B			
1	300	299.89	298.30	299.95	99.96	99.50
2	300	299.97	299.40	299.87	99.90	99.70
3	300	299.95	300.00	299.92	99.98	100.00
4	300	299.92	299.89	299.92	99.97	99.96

* Average of eight determinations. ** Recovery of amount added to the pharmaceutical formulation (average of three determinations).

Roxithromycin probably effects the reduction of one, two or three oxygen atoms from tungstate and/or molybdate, there by producing one or more of several possible reduced species which have a characteristic intense blue colour. In conclution, the proposed methods are simple, sensitive and accurate and can be used for the routine determination of roxithromycin in bulk as well as in its pharmaceutical preparations.

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Simultaneous Spectrophotometric Estimation of Isoniazid and Rifampicin from Combined Dosage Forms

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The present communication deals with the development of a simple, specific, sensitive, rapid and economical procedure for simultaneous estimation of rifampicin and isoniazid in a combined dosage form. The method is based on the native ultraviolet absobance maxima of the two chemotherapeutic agents. As both compounds do not interact chemically in phosphate buffered saline, two wavelengths 263 nm and 333 nm (λ_{max} of isoniazid and λ_{max} of rifampicin, respectively) were used. In addition, rifampicin also shows absorbance at 263 nm in phosphate buffered saline. Both the drugs obey Beer's law in the concentration range that was employed in the method.

Rifampicin (RIF) is a bactericidal antibiotic with a wide spectrum of activity. Though rifampicin is active against gram positive and gram negative bacteria, this drug has been advocated mainly for the treatment of tuberculosis[1]. Isoniazid (INH) is bacteriostatic for resting bacilli and chemically it is the hydrazide of isonicotinic acid i.e., 4-pyridine carboxylic acid hydrazide. RIF and INH are official in IP[2], BP[3,4] and USP[5,6]. A combination of 450 mg of RIF and 300 mg of INH is commercially available as tablets and capsules. The IP[7] suggests a microbiological method for RIF and a titrimetric method for INH. Few spectrophotometric methods reported in the literature for simultaneous estimation of RIF and INH are tedious and require number of steps and various types of reagents to get the final results [8,9]. The proposed method is specific, sensitive, rapid, economical and very simple to perform by using very few chemicals and steps.

A Shimadzu UV/Vis recording spectrophotometer (Model: UV-240, Graphiccord) with Spectral band width variable from 0.008 nm to 5 nm in 0.001 nm steps and a resolution of 0.1 nm with Photomultiplier detector R 928-05 was used in the study. The wavelength accuracy of the

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