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Utility of Dyes as Analytical Reagents for the Assay of Tylosin and Flunarizine

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ABSTRACT: Sensitive spectrophotometric methods (M_1 to M_3) for the assay of tylosin (TS) and flunarizine (FZ) in pharmaceutical formulations based on the formation of chloroform soluble ion-association complexes have been proposed. The utility of the three dyes namely Supracen violet 3B (SV 3B, M_1), Tropacolin 000 (TP 000, M_2) and Woolfast blue BL (WFB BL, M_3) in the formation of ion-association complex with TS and FZ has been discussed. The extracts of the ion-association complexes exhibit absorption maxima at 560, 480 and 580 nm for methods M_1, M_2 and M_3 respectively. The accuracy of the methods was checked by the comparison methods. These methods were found to be suitable for the assay of TS and FZ.

KEYWORDS: Dyes, Assay, Tylosin, Flunarizine.

INTRODUCTION

The ion-association complex is a special form of molecular complex resulting from two components extractable into organic solvents from aqueous phase at suitable pH. One component is a chromogen (dye) possessing charge and so insoluble in organic solvents. The other is colorless, possessing opposite charge to that of chromogen. Ion-association complex extraction has been applied to the estimation of numerous drugs possessing aliphatic tertiary amino groups[1] by using an acid dye as a reagent and a chlorinated solvent as an extractant. These methods are popular for their sensitivity or selectivity for the assay of drugs. Tylosin (TS)[2-4] is a macrolide antibiotic used in veterinary medicine in the prophylaxis and treatment of various infections caused by susceptible organisms. It is chemically known as ([4R-(4R*, 5S*, 6S*, 7R*, 9R*, 11E, 13E, 15R*, 16R*)]-15-[[6-deoxy-2,3-di-O-methyl-b-D-allo pyranosyl)oxy]methyl]-6-[[3,6-dideoxy-4-O-(2,6-dideoxy-3-C-methyl-a-L-ribo-hexopyrano- syl)-3-(dimethylamino)-b-D-glucopyranosyl]oxy]-16-ethyl-4-hydroxy-5,9,13-trimethyl-2,10-dioxooxacyclohexadeca-11,13diene-7-acetaldehyde)]. Flunarizine (FZ)as dihydro chloride,[4] is an antihistaminic, used as an inhibitor of central and peripheral vasoconstriction. It is chemically known as piperazine, 1-[bis (4-fluorophenyl) methyl]-4-[(2E)-3-phenyl-2-propenyl]-, dihydrochloride]. Literature survey reveals that there exist some visible spectrophotometric methods for the assay of TS[5-7] and FZ[8-12]. The present methods are based on the formation of ion-association complex involving tertiary amine of TS or FZ with the acidic dyes[13]. Supracen violet 3B (SV 3B), Tropacolin 000 (TP 000)[14] or Woolfast blue BL (WFB BL)[13]. The results for these methods were statistically validated.

MATERIALS AND METHODS

Instruments

Milton Roy Spectronic 1201 and Systronics 106 digital spectrophotometers with 1 cm matched quartz cells were used for the spectral and absorbance measurements. An Elico LI-120 digital pH meter was used for pH measurements.

Preparation of Drug solutions and Reagents

All the chemicals and reagents used were of analytical grade and the solutions were prepared in double distilled water.

Drug Solutions

TS: A 1 mg/ml solution was prepared by dissolving 100 mg of pure (98%) TS (for bulk samples) or active ingredient (for pharmaceutical formulations) in 100 ml of 0.1 M HCl. The stock solution was diluted step wise with the same solvent to obtain the working standard solutions of concentration 10 μg/ml (for method M_3) and 40 μg/ml (for methods M_1 and M_2).

FZ: A 1 mg/ml solution was prepared by dissolving

100 mg of pure (96%) FZ (for bulk samples) or active ingredient (for pharmaceutical formulations) in 100 ml of distilled water. The stock solution was diluted step wise with the same solvent to obtain the working standard solutions of 10 μg/ml (for method M_3), 20 μg/ml (for method M_2) and 40 μg/ml (for method M_1).

Dye Solutions

Aqueous solutions of 4.63 mM SV 3B (Chroma), 5.70 mM TP 000 (Fluka) and 3.26 mM WFB BL (Fluka) were prepared by dissolving the required amount in double distilled water.

Buffer and Acid Solutions:

The glycine- HCl buffer solution (pH 1.3 for method M_1 and pH 1.5 for method M_3) and HCl solution (E-Merck, 0.1 M for method M_2) were prepared.

Recommended Procedure

Aliquots of standard TS solution (1.0-5.0 ml, 40 μg/ml (M_1); 0.5-2.5 ml, 40 μg/ml (M_2); 1.0-6.0 ml, 10 μg/ml (M_3)) or standard FZ solution (1.0-6.0 ml, 40 μg/ml (M_1); 1.0-6.0 ml, 20 μg/ml (M_2); 1.0-6.0 ml, 10 μg/ml (M_3)) were taken into a series of 125 ml separating funnels. Now, 6.0 ml of buffer solution (pH 1.3 (M_1) and pH 1.5 (M_3) or 0.1 M HCl solution (M_2)) and 2.0 ml of SV 3B (M_1), TP 000 (M_2) or WFB BL (M_3) solutions were added. The total volume of aqueous phase in each separating funnel was adjusted to 15.0 ml with distilled water. To each separating funnel, accurately measured 5.0 ml of chloroform was added and the content was shaken for 2 min. The two phases were allowed to separate and the chloroform layer was collected. Now, 5 ml of chloroform was added again to the each of above flasks, the content was shaken again for 2 min. and the chloroform layer was collected. Then both of the chloroform layers were mixed (2x5 ml) and dried over anhydrous sodium sulphate. The absorbance of chloroform layer was measured at 560 nm (M_1), 480 nm (M_2) or 580 nm (M_3) against a reagent blank, which was similarly prepared. The concentrations of TS or FZ were deduced from the respective calibration curves.

Comparison Methods

TS: The UV spectrophotometric method which was suggested for the identification of TS in BP (Vet)[2] has been moulded for its assay and chosen as comparison method for ascertaining the accuracy of the proposed methods.

FZ: The visible spectrophotometric method reported earlier using Bromo cresol green[11] as a reagent has been chosen as the comparison method for ascertaining the accuracy of the proposed methods.

Results and Discussion

Parameters Fixation

The optimum conditions for the color development of methods were established by varying the one parameters at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species[15]. The following experiments were conducted for this purpose and the conditions so obtained were incorporated in the recommended procedures. Various parameters affecting the complex formation were studied systematically at room temperature. The effect of extracting solvent used was examined. The polarity of a solvent affects both extraction efficiency and absorbance intensity. Several organic solvents such as dichloromethane, chloroform, carbon tetrachloride, n-butanol, benzene, chlorobenzene and nitrobenzene were used for extracting the ion-association complex. Chloroform was selected because of its higher sensitivity and the considerably lower extraction of the dye. The effect of pH or acidity of the aqueous phase on the ion-association complex was studied. The color intensity of the chloroform extract reached a maximum when 6.0 ml of pH 1.3 buffer (M_1), 0.1 M HCl (M_2) or pH 1.5

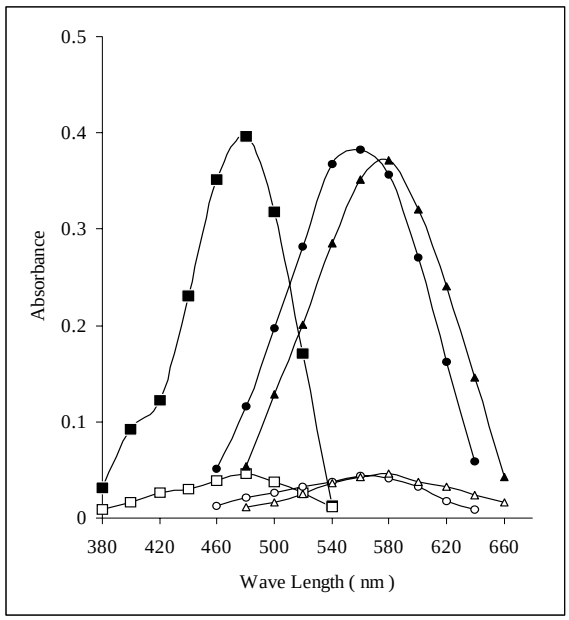

Fig 1. Absorption spectrum of TS-SV 3B System (•-•) against reagent blank (o-o) Vs distilled water (M_1), [TS] = 1.30 x 10^{-5} M, [SV 3B] = 9.26 x 10^{-4} M. Absorption spectrum of TS - TP 000 System (■-■) against reagent blank (□-□) Vs distilled water (M_2), [TS] = 6.54 x 10^{-6} M, [TP 000] = 1. 14 x 10^{-3} M. Absorption spectrum of TS – WFB BL System (▲-▲) against reagent blank (△-△) Vs distilled water (M_3),[TS]= 4.36 x 10^{-6} M, [WFB BL] = 6.52 x 10^{-4} M.

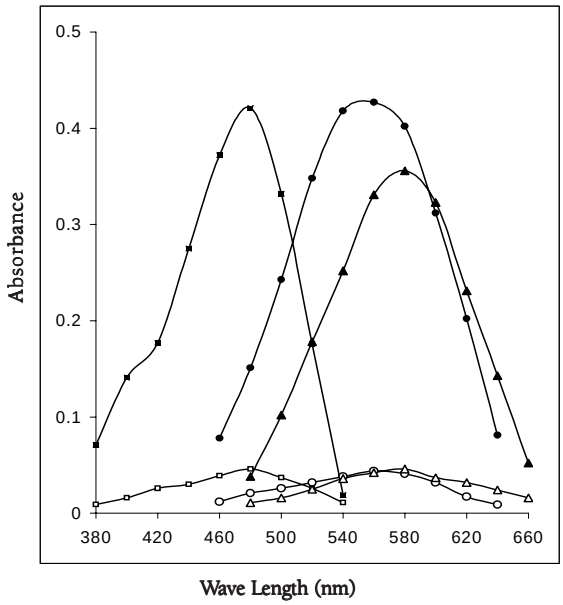

Fig 2. Absorption spectrum of FZ-SV 3B System (•-•) against reagent blank (o-o) Vs distilled water (M_1), [FZ] = 3.35 x 10^{-5} M, [SV 3B] = 9.26 x 10^{-4} M. Absorption spectrum of FZ - TP 000 System (■-■) against reagent blank (□-□) Vs distilled water (M_2), [FZ] = 1.67 x 10^{-5} M, [TP 000] = 1. 14 x 10^{-3} M. Absorption spectrum of FZ – WFB BL System (▲-▲) against reagent blank (△-△) Vs distilled water (M_3), [FZ]= 8.37 x 10^{-6} M, [WFB BL] = 6.52 x 10^{-4} M.

buffer (M_3) were used in the aqueous phase. Two ml of 4.63 mM SV 3B, 5.70 mM TP 000 or 3.26 mM WFB BL was necessary for covering the broad range of Beer's law limits. A ratio of 3:2 aqueous to organic phase was required for the efficient extraction of the complex. Shaking times ranging from 0.5 to 5.0 min did not produce any change in the color intensity and so 2 min shaking time was selected. The color products were stable upto 30 min and were determined at 560, 480, 580 nm for methods M_1, M_2 and M_3 respectively (Figs. 1 and 2).

Analytical Data

In order to test whether the colored species formed in the above methods adhere to Beer's law, the absorbances of a set of solutions containing varying amounts of TS or FZ and specified amounts of reagents (as given in the recommended procedures) were recorded at appropriate wavelengths against the corresponding reagent blanks. Beer's law limits, molar absorptivity, the Sandell sensitivity and working photometric range for TS and FZ in each method developed with mentioned reagents were calculated. Least square regression analysis was carried out for getting the slope, intercept and the correlation coefficient values. The precision of the proposed methods was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of TS in total solution (12 μg/ml, M_1; 6 μg/ml, M_2; and 4 μg/ml, M_3) or FZ in total solution (16 μg/ml, M1; 4 μg/ml, M_2; and 4 μg/ml, M_3). The percent relative standard deviation and percent range of error (95% confidence level) were calculated for the proposed methods (Table 1).

Interference Studies

The effect of various substances that often accompany TS and FZ in various pharmaceutical formulations were studied separately in all the methods. To an aliquot containing 120 μg (M_1), 60 μg (M_2) and 40 μg (M_3) of TS or 160 μg (M_1), 40 μg (M_2) and 40 μg (M_3) of FZ, different amounts of various ingredients and additives were added individually until a solution showed the same absorbance (± 0.01) as that of pure TS or FZ solution under experimental conditions as

Table 1. Optical characteristics, precision and accuracy of the proposed methods for tylosin and flunarizine.

Parameters	Tylosin			Flunarizine		
	M_1	M_2	M_3	M_1	M_2	M_3
λ_{max} (nm)	560	480	580	560	480	580
Beer's law limits (μg / ml)	4-20	2-10	1-6	4-24	1-6	1-6
Molar absorptivity (1 mol^{-1} cm^{-1})	2.87×10^4	6.03×10^4	8.97×10^4	1.27×10^4	2.50×10^4	4.21×10^4
Sandell's Sensitivity (mg cm^{-2} / 0.001 absorbance unit)	0.031	0.015	0.004	0.037	0.019	0.011
Optimum photometric range (μg / ml)	5.6-18.9	2.9-8.9	1.4-5.6	5.6-19.9	3.8-12.4	1.6- 3.9
Regression equation (y)*						
Slope (b)	3.13×10^{-2}	6.56×10^{-2}	9.24×10^{-2}	2.66×10^{-2}	5.26×10^{-2}	8.71×10^{-2}
Intercept (a)	3.90×10^{-3}	2.00×10^{-4}	3.46×10^{-3}	-2.66×10^{-2}	-1.00×10^{-3}	5.73×10^{-3}
Correlation coefficient (r)	0.9999	0.9998	0.9999	0.9999	0.9999	0.9999
Relative standard deviation (%) **	0.418	0.470	1.145	0.941	0.812	0.861
% range of error (95 %confidence level)	0.438	0.494	1.201	0.988	0.853	0.903

*y = a + bc, where c is the concentration in mg/ml and y is the absorbance unit.
**Six replicate samples.

described under the procedure. The commonly used concomitants and additives in the preparation of formulation such as talc (upto 200-fold (excess) (w/v), starch (150 fold), boric acid (150 fold), stearic acid (70 fold), magnesium stearate (50 fold), kaolin (30 fold), sodium lauryl sulfate (10 fold) and gelatin (10 fold), did not interfere with the assay of TS or FZ by proposed methods.

Analysis of Formulations

Commercial formulations (tablets and injections or capsules) containing TS or FZ were successfully analyzed by the proposed methods. The values obtained by the proposed and comparison methods for the formulations were compared statistically with t-and F-tests and found not to differ significantly. The results were summarized in Table 2.

Chemistry of the Colored Species

TS or FZ being basic in nature forms ion-association complex with the acidic dye namely SV 3B[13], TP 000[14] or WFB BL[13], which is extractable into chloroform. The protonated aliphatic tertiary nitrogen (positive charge) of the TS or FZ in acidic medium is expected to attract the oppositely charged part (negative charge) of the dye (SO_3^-) and behave as a single unit being held together by an electrostatic attraction. The possible structure of the ion-association complex in each method was established (as given in schemes 1 and 2) and was further confirmed by slope analysis method.

Conclusions

In the present study TS and FZ were assayed successfully in bulk and in pharmaceutical formulations. The ingredients usually present in pharmaceutical formulations do not interfere in the proposed methods. Thus the proposed methods are simple and sensitive with good precision and accuracy for the assay of TS and FZ in pharmaceutical formulations in quality control laboratories.

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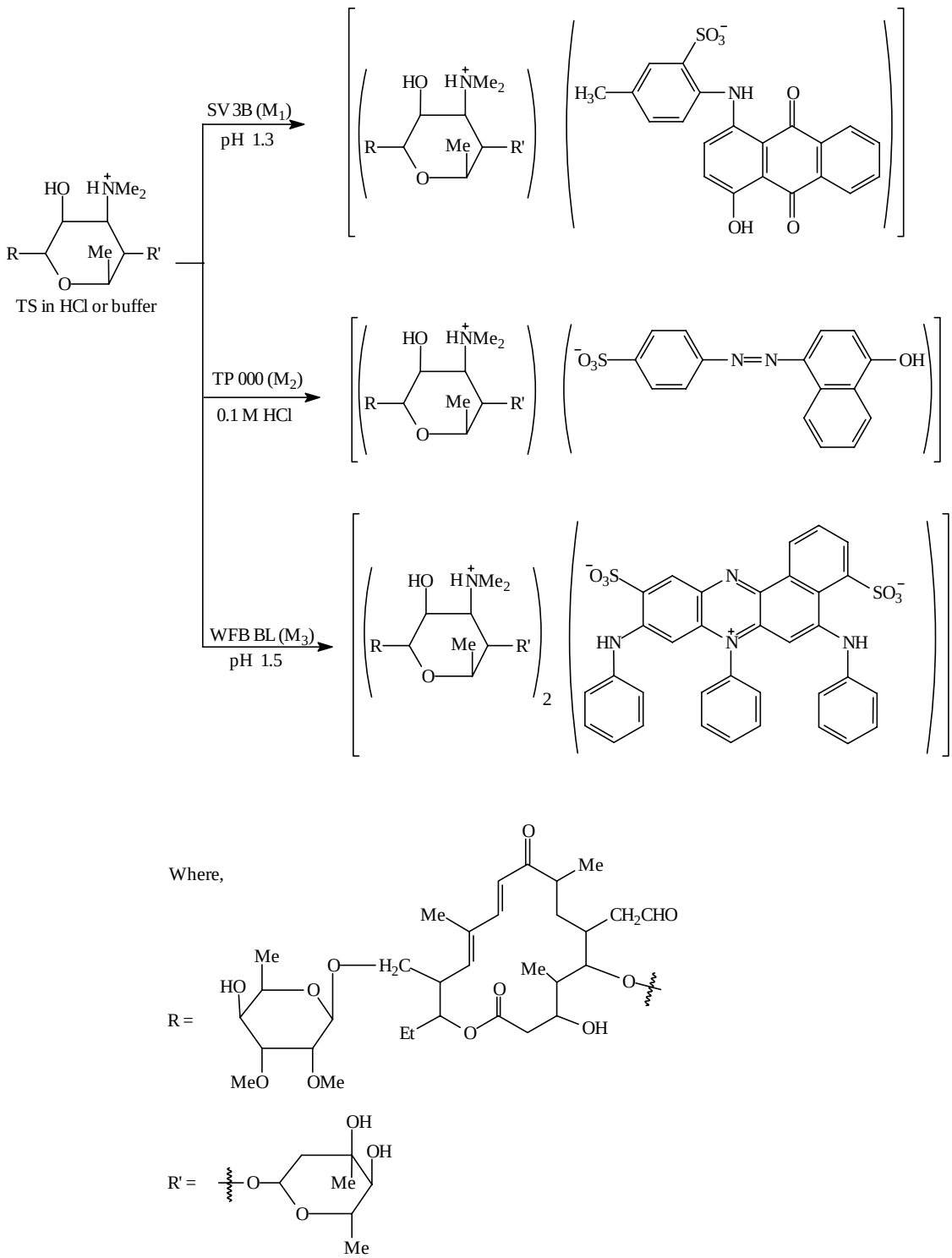
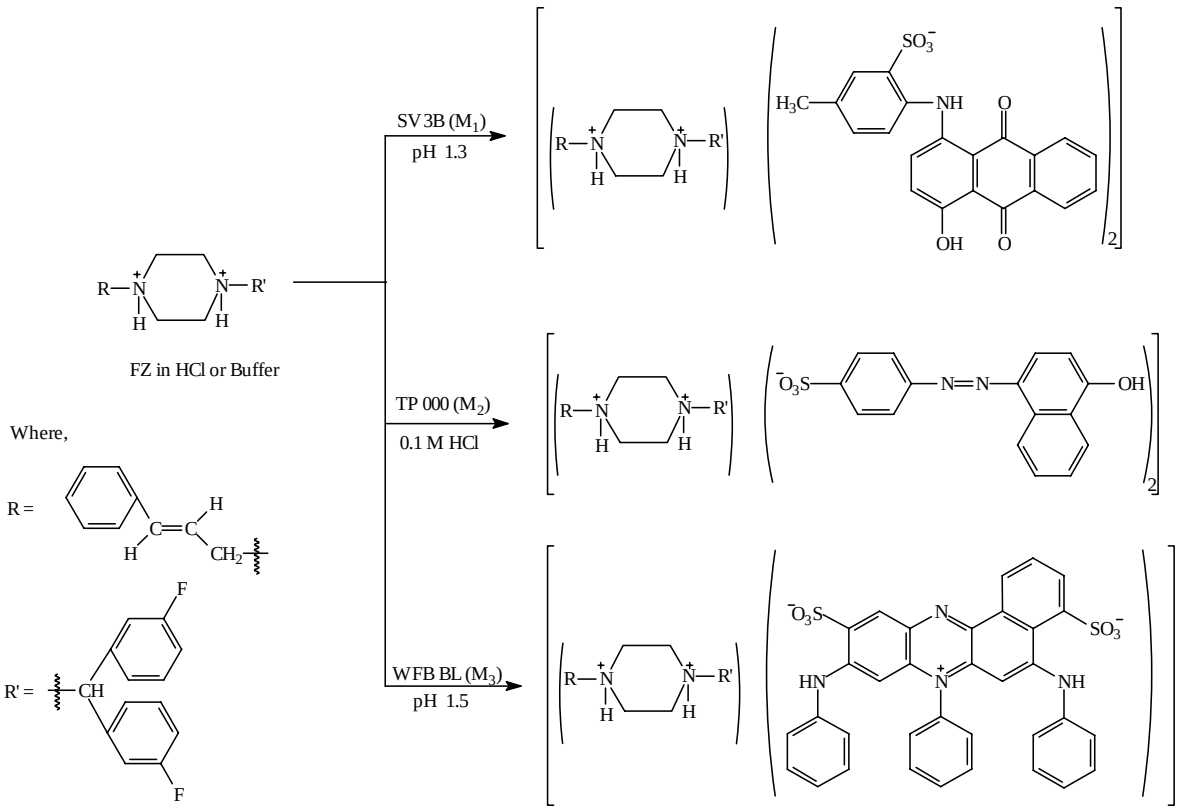
Table 2. Assay of tylosin and flunarizine in pharmaceutical formulations.

Pharmaceutical formulations*	Labeled amount	% recovery by proposed methods**			Comparison method
		M_1	M_2	M_3	
Trazodone:					Ref 2
Tablets – T_1	200 mg	99.05 ± 0.26	99.76 ± 0.21	99.76 ± 0.21	99.51 ± 0.25
	t = 1.01	t = 1.06	t = 1.06		
	F = 1.08	F = 1.41	F = 1.41		
Tablets – T_2	200 mg	99.93 ± 0.27	100.23 ± 0.21	99.92 ± 0.23	99.92 ± 0.20
	t = 0.45	t = 1.33	t = 0.28		
	F = 1.82	F = 1.10	F = 1.32		
Injections – I_1	50 mg/ml	99.82 ± 0.46	99.96 ± 0.35	99.80 ± 0.56	99.56 ± 0.45
	t = 1.81	t = 0.66	t = 0.60		
	F = 1.04	F = 1.65	F = 1.54		
Injections – I_2	50 mg/ml	99.82 ± 0.54	99.93 ± 0.70	99.98 ± 0.66	99.83 ± 0.50
	t = 0.34	t = 0.23	t = 0.23		
	F = 1.16	F = 1.96	F = 1.74		
Flunarizine:					Ref 11
Tablets – T_1	5 mg	99.61 ± 0.50	99.18 ± 0.25	99.25 ± 0.62	99.76 ± 0.32
	t = 0.38	t = 1.02	t = 1.86		
	F = 2.44	F = 1.63	F = 3.75		
Tablets – T_2	10 mg	99.42 ± 0.42	99.19 ± 0.38	99.22 ± 0.45	99.86 ± 0.26
	t = 0.91	t = 1.22	t = 1.13		
	F = 2.60	F = 2.13	F = 2.99		
Capsules – C_1	5 mg	99.81 ± 0.33	99.27 ± 0.45	99.16 ± 0.38	99.26 ± 0.49
	t = 2.01	t = 1.75	t = 1.23		
	F = 2.20	F = 1.18	F = 1.66		
Capsules – C_2	10 mg	99.17 ± 0.42	99.82 ± 0.60	99.62 ± 0.27	99.76 ± 0.38
	t = 0.80	t = 1.21	t = 1.10		
	F = 1.22	F = 2.49	F = 1.98		

* Two different batches each of formulations from a pharmaceutical company.
** Average of six determinations; the t- and F- values refer to comparison of the proposed method with the comparison method. Theoretical values at 95% confidence limits, t =2.57, F =5.05.



Scheme 1. Ion-association complexes of TS with SV 3B, TP OOO and WFB BL.



Scheme 2. Ion-association complexes of FZ with SV 3B, TP 000 and WFB.

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