

Research Article

Extractive Spectrophotometric Methods for the Determination of Metoprolol Succinate in Pure and Pharmaceutical Formulations

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Abstract

Two simple, rapid and sensitive extractive spectrophotometric methods have been developed for the assay of metoprolol succinate (MPS) in pure and pharmaceutical formulations. These methods were based on the formation of chloroform soluble ion-association complexes of MPS with bromocresol green (BCG) in NaOAc-HCl buffer of pH 3.29 [Method A] and with bromophenol blue (BPB) in KCl-HCl buffer of pH 2.2 [Method B]. The coloured products have absorption maxima at 415 nm and 412 nm for method A and method B, respectively. The coloured products obeyed Beer's law in the concentration ranges of 0.2-8.2 and 0.1-6.5 μgml^{-1} for methods A and B, respectively. The molar absorptivity values as obtained from Beer's data were found to be 5.6×10^4 and 7.01×10^4 $\text{l.mol}^{-1}\text{cm}^{-1}$ while Sandell's sensitivity values were calculated to be 11.64 and 9.30 ng cm^{-2} for method A and method B, respectively. No interference was observed from common excipients present in pharmaceutical formulations. The methods were successfully applied for the analysis of pharmaceutical formulations.

Keywords: Metoprolol succinate; Spectrophotometry; Assay; Ion-association; Formulations

Introduction

Drugs are important sources to relieve the pain and cure diseases when they are in proper and appropriate dosage form. Hence, it is very essential to control and monitor the quality of the synthesized bulk drug as well as the formulations accurately. Analytical chemistry in particular, helps in the quality assurance and control of bulk drug and their dosage forms [1,2]. The pharmaceutical industries employ various analytical techniques to determine not only the active ingredient (s) but also the quantification of related compounds or impurities in drug as well as formulations. This is essential to reduce the side effects from impurities and to increase the efficiency of drug molecule. The assay and purity of drug samples in bulk and formulations may be determined by means of physical, chemical, physico-chemical and biological methods [1-8]. Physical and physico-chemical methods are simple and more commonly employed. The literature methods for the analysis of drugs often need improvement to suit laboratory requirements and upgradation of facilities available in a particular laboratory set up. The modern and advanced methods of analysis (HPLC, GLC, NMR and Mass) offer good speed, precision and accuracy, but they involve sophisticated equipments which are costly and pose problems of maintenance [1,3]. Hence, small scale industries, which produce bulk drugs and pharmaceutical formulations, are not able to utilize those advanced methods of analysis. Among various analytical techniques, spectrophotometry still plays a significant role in the determination of compounds at micro or nanogram levels [2]. It is simple, economically viable and easy to carry-out. The importance of a spectrophotometric method lies in the chemical reaction(s) upon which the procedures are

based, rather than upon the sophistication of the instrument. Many reactions which absorb UV radiation or yield colored species for a particular drug are found to be selective or can be rendered selective by controlling and optimizing the reaction conditions. Hence, spectrophotometry is generally preferred in small scale industries and most of the laboratories for routine quality assurance [9].

Metoprolol succinate (MPS), chemically designated as (\pm)-1-(isopropylamino)-3-[P (2-methoxyethyl) phenoxy]-2-propanol succinate, is a kind of β adrenaline receptor blocker. It is widely used for the treatment of hypertension, angina, myocardial infarction, arrhythmia, hyperthyroidism and other related diseases [10-12]. It is so sensitive that even a small oral dose of the drug gives sufficient blockade. Since the β - blockers are misused as doping agents in sports, these drugs have been added to the list of forbidden drugs by the International Olympic Committee [13]. It is official in various pharmacopoeias [10-12]. In view of its biological importance, several analytical methods have been reported for quantitative determination of MPS [14-25] which include gas chromatographic [14-15], high-performance liquid chromatographic [16-18] and spectrophotometric methods [19-25].

The reported spectrophotometric methods suffer from different limitations *viz.*, less sensitivity [19-24], work only at higher concentration of the drug [19,24], require heating at 70°C for 10min [22] and long standing [21,23,25]. In view of this, it was planned to develop sensitive spectrophotometric methods for the determination of MPS in bulk and pharmaceutical formulations. The proposed extractive spectrophotometric methods are based on the formation of chloroform soluble ion-association complexes of MPS with

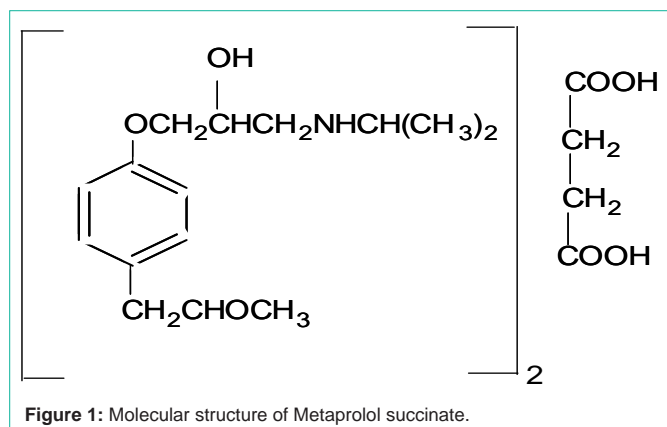


Figure 1: Molecular structure of Metoprolol succinate.

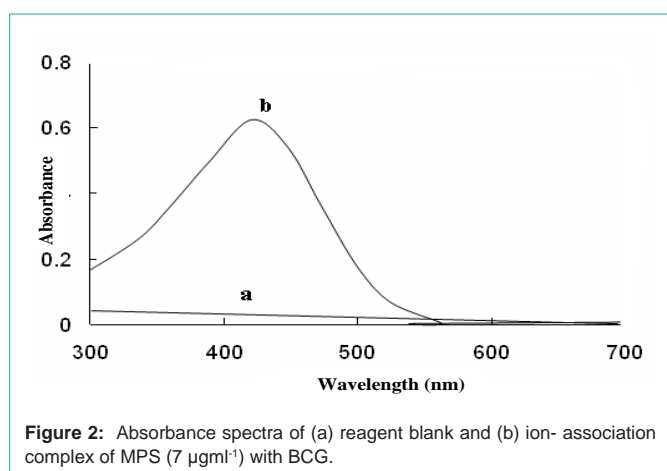


Figure 2: Absorbance spectra of (a) reagent blank and (b) ion- association complex of MPS ($7 \mu\text{gml}^{-1}$) with BCG.

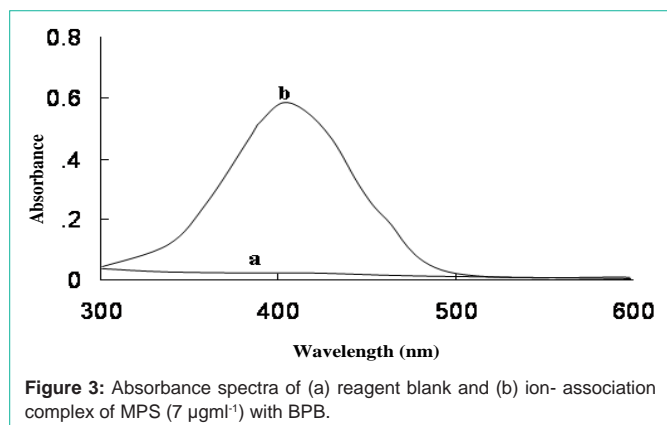


Figure 3: Absorbance spectra of (a) reagent blank and (b) ion- association complex of MPS ($7 \mu\text{gml}^{-1}$) with BPB.

bromocresol green (BCG) and bromophenol blue (BPB) in acidic buffer. These methods are more sensitive and simple compared to reported spectrophotometric methods.

Experimental

Reagent preparation

Standard drug solution: A stock solution of MPS containing $100 \mu\text{gml}^{-1}$ was prepared in distilled water and diluted as and when required. The solution was stable at room temperature.

Preparation of dye solution: 0.5 % (w/v) of bromocresol green (BCG) and bromophenol blue (BPB) were prepared by dissolving

500 mg each in distilled water and volume made up to 100 ml with distilled water.

Buffers: Different buffers were prepared following the standard methods reported earlier [26-28].

Recommended procedures

In order to know the effect of various parameters involved in the formation of coloured products (as described under results and discussion), a detailed study was carried out and the following procedures were recommended for the assay of MPS in pure and pharmaceutical formulations.

Assay procedure for pure drug: Suitable amounts of aliquot of pure MPS solution containing $0.2\text{--}8.2 \mu\text{gml}^{-1}$ for method A or $0.1\text{--}6.5 \mu\text{gml}^{-1}$ for method B were transferred into a series of 125ml of separatory funnels. A volume of 3 ml of NaOAc-HCl buffer of pH 3.29 for method A or 3ml of KCl-HCl buffer of pH-2.2 for method B and 5ml of BCG or 7ml of BPB were added to each separatory funnel. It was diluted to 25ml with distilled water. Chloroform (10 ml) was added to each of the separating funnels; the contents were shaken well and allowed to stand at room temperature for 2min for the separation. The two phases were allowed to separate and the chloroform layer was passed through anhydrous sodium sulphate and transferred to 10ml volumetric flask, then the extract was made up to the mark with chloroform and mixed well. The absorbance of yellow coloured complexes were recorded at 415nm and 412nm against the corresponding reagent blank for method A and method B, respectively. A calibration graph was constructed.

Assay of pharmaceutical preparations: Ten tablets were weighed and powdered. An amount of the powder equivalent to 25mg of MPS weighed accurately and transferred into a 100ml beaker. Using the mechanical stirrer, the powder was completely disintegrated in distilled water. The solution was filtered through a Whatman filter paper number 40 to remove the insoluble matter. The filtrate was transferred into a 100ml volumetric flask and diluted to the mark with distilled water. Suitable volume of the filtrate was further diluted as and when required. Appropriate aliquot was taken and analyzed using the procedure given above.

Results and Discussion

Metoprolol succinate (MPS) is a white crystalline powder having molecular formula $\text{C}_{34}\text{H}_{56}\text{N}_2\text{O}_{10}$. It is practically insoluble in ethyl acetate, very slightly soluble in dichloromethane and soluble in water and methanol. It is a beta 1-selective (cardio selective) adrenergic receptor blocking agent. Its structure is given in Figure 1.

Extractive spectrophotometric procedures are popular for their sensitivity in the assay of drugs. Hence, ion-pair extractive spectrophotometry has received a considerable attention for the quantitative determination of many pharmaceutical compounds [29-30]. Secondary amine group of MPS undergoes protonation in acidic medium. Anionic dyes such as BCG and BPB form ion-association complexes with the positively charged drug. The drug to dye stoichiometric ratio as calculated by the Job's method of continuous variation [31] was found to be 1:1 with BCG/BPB. Each drug-dye complex, with two oppositely charged ions behaves as a single unit held together by an electrostatic force of attraction.

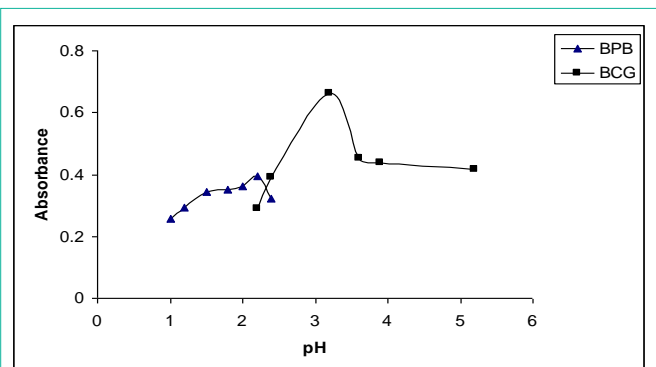
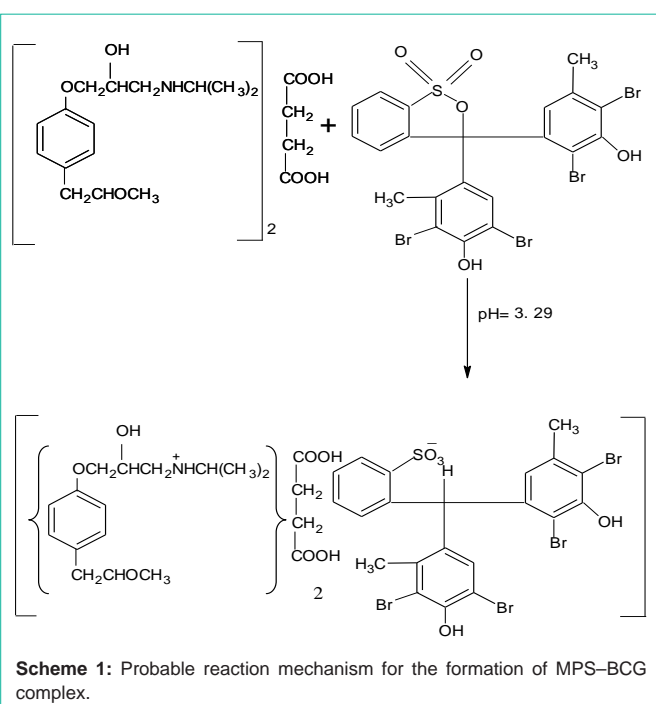


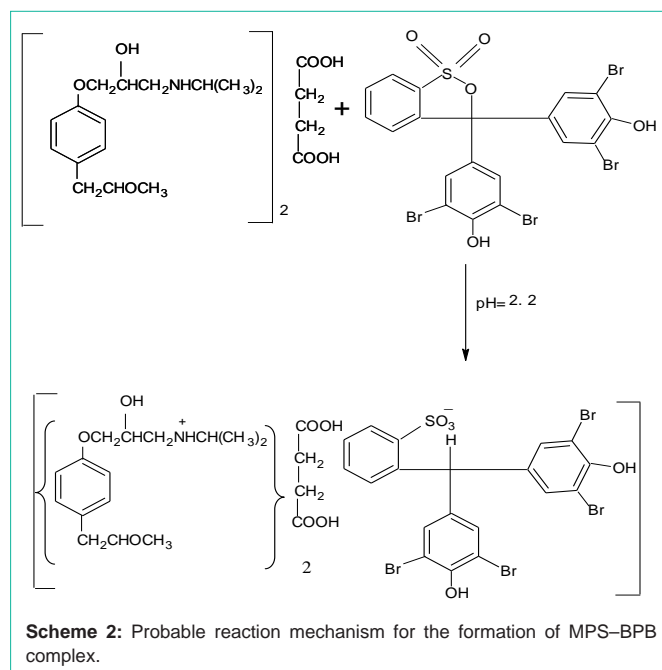
Figure 4: Effect of pH of the buffer on absorbance of the ion-association complexes of MPS with BCP and BPB.



The MPS reacted with BCP and BPB in acidic buffer to form chloroform- soluble yellow coloured 1:1 ion-association complex, which exhibited absorption maxima at 41nm (Figure 2) and 412nm (Figure 3) for method A and method B, respectively. Under similar experimental conditions, the reagents blank showed negligible absorbance thereby permitting good analytical conditions for quantitative determination of MPS. The probable reaction mechanism for the formation of ion-pair complexes MPS-BCG and MPS-BPB are given in Schemes 1 and Schemes 2.

Fixation of parameters

The optimum reaction conditions were established by varying the parameter one at a time keeping the others constant and the effects produced on the absorbances of the coloured species [32] were observed. The effects of various parameters for both methods *viz.*, the pH of buffer solution, the amount of buffer solution, the concentration of dye (BCG or BPB), the amount of dye, choice of extractant and effect of temperature on the coloured complexes were investigated.



Effect of buffer/ pH: The effect of pH was studied by extracting the coloured complexes in presence of various buffers such as KCl-HCl (pH=1.0-2.2), NaOAc-HCl (pH=1.99-4.92), NaOAc-AcOH (pH=3.72-5.57) and potassium hydrogen phthalate-HCl (pH= 2.2-3.6). It was noticed that the maximum colour intensity and constant absorbances were observed in NaOAc-HCl of pH 3.29 for BCP and in KCl-HCl buffer (Clark and Lubs) of pH 2.2 for BPB (Figure 4). Further, 3 ml of NaOAc-HCl of pH 3.29 for BCP and 3ml of KCl-HCl buffer of pH 2.2 for BPB gave maximum absorbances and reproducible results. Low absorbance values were observed at pH values higher or lower than the stated above for BCP and BPB in the corresponding buffer medium. Hence, NaOAc-HCl buffer of pH 3.29 and Clark and Lubs buffer of pH 2.2 for both methods were selected for all subsequent measurements.

Effect of reagent: In order to examine the effect of the reagents (BCP and BPB), the absorbances of solutions containing a fixed concentration of MPS and different amounts of the respective reagent were measured separately. It was noticed that the constant and maximum absorbance values were obtained with 3.0 ml of 0.5% BCP or 3.0ml of 0.5% BPB (Figure 4). The absorbances decreased with increase in concentration of the corresponding reagent.

Choice of extractant: Several organic solvents *viz.*, chloroform, carbon tetrachloride, ethyl acetate, xylene, diethylether, butyl acetate, toluene, dichloromethane and chlorobenzene were tried for effective extraction of the coloured species from aqueous phase. Only partial extraction of the complex was achieved with solvents other than chloroform. Chloroform was found to be the most suitable extractant as it was observed that only one extraction was adequate to achieve a quantitative recovery of the complex. Shaking times of 0.5 to 2 min produced constant absorbances and hence a shaking time of 1min was maintained throughout. There was no appreciable change in the absorbance or colour of the product even if the order of addition of the reactants was varied.

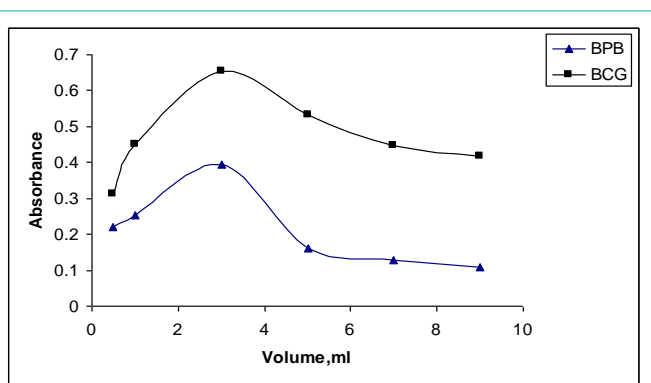


Figure 5: Effect of reagent on absorbance of the ion-association complexes of MPS with BCG and BPB.

Table 1: Optical characteristics, precision and accuracy data.

Parameters	Values for	
	BCG	BPB
λ_{\max} (nm)	415	412
Beer's law limits (μgml^{-1})	0.2-8.2	0.1-6.5
Molar absorptivity ($\text{l/mol/cm} \times 10^4$)	5.6	7.01
Sandell's sensitivity (ng cm^{-2})	11.64	9.302
Correlation co-efficient (r)	0.9995	0.9990
Regression equation (Y) ^a	-----	-----
Slope (b)	0.0845	0.0965
Intercept (a)	0.0068	0.096
Relative standard deviation (%) ^d	0.82	0.93
% Error ^d	1.09	1.35
Limit of detection (μgml^{-1})	0.057	0.027
Limit of quantification (μgml^{-1})	0.19	0.09

^a $Y=a+bX$, where X is the concentration of the drug in μgml^{-1} .

^d Average of five determinations.

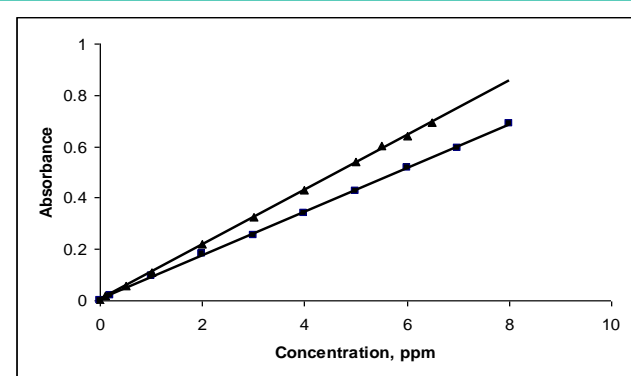


Figure 6: Beer's law plot of MPS for and BPB. (▲) and BCG (■).

Effect of temperature on the coloured complexes: The effect of temperature on coloured complexes was studied at different temperatures. The coloured complexes were found to be stable up to 34°C. At higher temperatures, the absorbances of the coloured complexes increased due to volatile nature of chloroform. However, the complexes were stable for more than 1.5h at room temperature.

Spectral characteristics

Absorption spectra of yellow coloured MPS-BCG and MPS-BPB complexes are shown in Figure 2 and Figure 3. The corresponding reagents blank exhibited practically negligible absorbances thereby permitting good analytical conditions for quantitative determination of MPS.

Detection and quantification limits: The LOD values were found to be 0.057 and 0.027 μgml^{-1} while those of LOQ values were observed to be 0.19 and 0.09 μgml^{-1} for MPS with BCG and BPB, respectively. These values indicated that the BPB method was more sensitive compared to BCG method. In general, low values of LOQ revealed good accuracy of the proposed methods (Figure 5).

Quantification: The Beer's law limits, molar absorptivity and Sandell's sensitivity values were evaluated (Table 1). Regression analyses of Beer's law plots at their respective λ_{\max} values revealed a good correlation. Graphs of absorbance versus concentration showed almost zero intercept, and are described by regression equation, $Y=a+bX$ (where Y is the absorbance of a 1cm layer, b is the slope, a is the intercept and X is the concentration of drug in μgml^{-1}) obtained by least-squares method. The results are summarized in Table 1.

Precision and accuracy: The precision and accuracy of the proposed methods were checked by analyzing five replicates of the drug within Beer's law limits. The low percentage error and RSD values highlighted good accuracy and precision of the proposed methods (Table 1, Figure 6).

Recovery studies: Recovery studies were carried out by standard addition method. For this, known quantities of pure MPS were mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed as before. The total amount of the drug was then determined and the amount of the added drug was calculated by difference. The average percent recoveries obtained were quantitative (99.55-100.23%), indicating good accuracy of the methods.

Interference studies: The effects of common excipients and additives viz., starch, glucose, dextrose, lactose, magnesium stearate, gum acacia and talc (at the levels generally present in pharmaceutical formulations) were tested for their possible interferences in the assay of MPS and the results are shown in Table 2, for a representative dye, BPB. It is evident that the excipients and additives did not interfere in the determination of MPS.

Ruggedness: To ascertain the ruggedness of the methods, five

Table 2: Determination of MPS in presence of excipients and additives using BPB.

Excipients added	Amount (mg) ^a	% Recovery of MPS \pm %RSD ^b
Starch	30	99.3 \pm 1.02
Glucose	40	100.4 \pm 0.92
Dextrose	40	99.7 \pm 0.96
Lactose	40	101.0 \pm 0.98
Magnesium stearate	30	99.2 \pm 0.95
Talc	40	99.4 \pm 1.10
Gum acacia	40	99.8 \pm 0.86

^a 4 $\mu\text{g ml}^{-1}$ of MPS was taken.

^b Average of five determinations.

Table 3: Between-day precision of the assay of MPS by proposed methods.

Formulation	Label claim (mg)	Amount (found in mg)*		RSD%	
		BCG	BPB	BCG	BPB
Seloken-XL ^a	25	24.88	24.92	0.82	0.91
	50	50.20	50.48	0.58	0.46
	100	99.73	100.08	0.20	0.32
Metolar ^b	25	24.82	24.86	0.26	0.41
	50	50.32	49.88	0.32	0.52
	100	100.30	99.76	0.44	0.24
Meto-FR ^c	25	24.80	25.03	0.17	0.23
	50	49.92	50.61	0.61	0.56
	100	99.36	100.08	0.44	0.37

*Average of five determinations.

^aAstra Zeneca, India, Ltd.

^bCipla pharmaceuticals Ltd.

^cMono pharmaceuticals Ltd.

Table 4: Determination of MPS in pharmaceutical preparations by the proposed methods and their comparison with official method [10].

Formulation	Label claim (mg)	%Recovery * ± %RSD		
		Official method ¹	BCG	BPB
Seloken-XL ^a	25	99.89 ± 0.69	99.74 ± 0.62 F = 1.23; t = 1.42	99.81 ± 0.66 F = 1.13; t = 1.64
	50	99.84 ± 0.59	98.96 ± 0.54 F = 1.19; t = 1.54	99.35 ± 0.62 F = 1.31; t = 1.56
	100	98.92 ± 0.84	98.69 ± 0.66 F = 1.61; t = 1.48	99.02 ± 0.53 F = 1.50; t = 1.56
Metolar ^b	25	99.53 ± 0.74	99.78 ± 0.63 F = 1.38; t = 1.64	99.68 ± 0.72 F = 1.30; t = 1.71
	50	99.28 ± 0.56	99.38 ± 0.79 F = 1.99; t = 1.69	99.41 ± 0.94 F = 1.41; t = 1.77
	100	98.86 ± 0.79	98.98 ± 0.74 F = 1.14; t = 1.56	98.59 ± 0.64 F = 1.33; t = 1.58
Meto-FR ^c	25	99.25 ± 0.74	99.38 ± 0.61 F = 1.47; t = 1.72	99.41 ± 0.68 F = 1.24; t = 1.56
	50	99.75 ± 0.68	99.36 ± 0.86 F = 1.59; t = 1.71	99.45 ± 0.77 F = 1.24; t = 1.53
	100	99.48 ± 0.46	99.12 ± 0.51 F = 1.23; t = 1.24	99.54 ± 0.74 F = 2.10; t = 1.36

*Average of five determinations.

Tabulated t- value of 95% confidence limit level is 2.78 for n = 5.

Tabulated F- value of 95% confidence limit level is 6.39 for n = 5.

replicate determinations at different concentration levels of the drug were carried out. The within-day assay RSD values were found to be less than 1.15%. The values of between-day assay RSD for different concentrations of drug are given in Table 3. These values revealed that the proposed methods have reasonable ruggedness.

Analysis of pharmaceutical formulations and statistical comparison of the results with official method [10]

The proposed methods were successfully applied to the analysis of MPS in tablets. The results of analysis of pharmaceutical formulations (Table 4) were compared statistically by Student t-test and by the variance ratio F-test with those obtained by official method [10]. The Student t-values at 95% confidence level did not exceed the theoretical value indicating that there was no significant difference between the proposed and official methods. It was also observed that the variance ratio F-values calculated for p=0.05 did not exceed the theoretical value indicating that there was no significant difference between the precision of the proposed and reported methods.

Conclusions

In conclusion, the proposed methods are advantageous when compared to the reported methods. Unlike the gas chromatographic and HPLC procedures, the instrument was simple and was not of

high cost. The importance lies in chemical reactions upon which the procedures are based, rather than upon the sophistication of the instrument. This aspect of spectrophotometric analysis was of major interest in analytical pharmacy since it offers distinct possibility in the assay of a particular component in complex formulations. The reagents utilized in the proposed methods are cheaper, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. The proposed methods are free from interference by common additives and excipients. The wide applicability of the new procedures for routine quality control is well established by the assay of MPS in pure form and in pharmaceutical preparations. Hence, the methods could be employed as better alternatives to existing and official methods.

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