Optimized and Validated Spectrophotometric Methods for the Determination of Verapamil Hydrochloride in Drug Formulations

Nafisur Rahman*, **Nadeem Ahmad khan and Syed Najmul Hejaz Azmi**

Department of Chemistry, Aligarh Muslim University, Aligarh-202002 (U.P.) India.

* Corresponding author, E-mail: cht17nr@yahoo.co.in

ABSTRACT: Two spectrophotometric methods have been described for the assay of verapamil hydrochloride in drug formulations. Method A is based on the oxidation of the drug with potassium metaperiodate in sulfuric acid medium to give colored product, which absorbs maximally at 425 nm. Method B involves the formation of colored chloroform extractable ion-pair complex of the drug with tropaeolin 000 No.1 at pH 4.0 showing absorption peak at 400 nm. Under the optimized experimental conditions, Beer's law is obeyed in the concentration ranges of 12.5-187.5 and 2.0-30.0 μg mL^{-1} for methods A and B, respectively. Results of analyses were validated statistically and through recovery studies. Statistical comparison of the results with the reference method shows excellent agreement and indicates no significant difference in accuracy and precision. Both methods have been successfully applied to the determination of verapamil hydrochloride in drug formulations.

KEYWORDS: verapamil hydrochloride, potassium metaperiodate, tropaeolin 000 No.1, drug formulations, validation.

INTRODUCTION

Verapamil hydrochloride i.e. ($\pm$)-5-[N-(3,4-dimethoxyphenethyl) methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile monohydrochloride is clinically a very useful member of the calcium channel blocker. It is used in the treatment of supraventricular arrhythmias, angina and hypertension.[1-2]

The drug and its formulations are officially listed in British Pharmacopoeia[3] which suggests potentiometric titration method, The United States Pharmacopoeia[4] recommends a gas chromatographic method and The Indian Pharmacopoeia[5] describes a non-aqueous titration method for its assay in bulk and dosage forms. The different analytical methods that are reported for its determination include high performance liquid chromatography,[6] high performance thin layer chromatography,[7] liquid chromatography,[8] gas chromatography,[9] potentiometry-conductometry,[10] stripping voltammetry,[11] and atomic emission spectrometry.[12] Although the above methods have adequate sensitivity to assay lower concentrations of the drug and hence use of these methods are justified when the concentration of drug in biological fluids is low. The drug concentrations (40-240 mg per tablet) are high enough in pharmaceutical formulations. Therefore, the need for spectrophotometric method is fully justified.

A review of literature revealed that few spectrophotometric methods for the assay of verapamil hydrochloride based on charge transfer complexes with 2,3,5,6-tetrachloro-p-benzoquinone,[13] 2,3-dichloro-5,6-dicyano p-benzoquinone and 2,5-dichloro-3,6-dihydroxy-p-benzoquinone,[14] chromotrope 2B and chromotrope 2R azodyes,[15] extractable ion-pair complexes with bromothymol blue, bromophenol blue, bromocresol green, bromocresol purple and methyl orange.[16] However, a simple spectrophotometric procedure based on the reaction of the drug with an oxidant is not known.

This paper describes two spectrophotometric methods for the assay of verapamil hydrochloride in pure and dosage forms. Method A is based on the oxidation of drug with potassium metaperiodate in sulfuric acid medium. Method B utilizes the formation of an ion-pair complex of the drug with tropaeolin 000 No.1 and subsequent extraction of the yellow color into chloroform under optimized reaction conditions used. The proposed methods are optimized and validated as per the International conference on Hormonisation (USA) guidelines.[17]

MATERIALS AND METHODS

Apparatus

A Milton Roy Spectronic 20D$^+$ spectrophotometer (USA) and an Elico model L1-10 (New Delhi, India) pH meter were used for absorbance and pH measurements, respectively. A water bath shaker (NSW 133, New Delhi, India) was used to control the heating temperature for color development.

Reagents and Standards

All chemicals and reagents used were of analytical grade or pharmaceutical grade. All solutions were prepared in doubly distilled water. Aqueous solutions of potassium metaperiodate (0.2%; Otto Chemie, Mumbai, India), tropaeolin 000 No.1 (0.05%, Fluka Chemie AG, Switzerland), and 10 M sulfuric acid (Merck Limited, Mumbai, India) were prepared.

A buffer solution of pH 4 was prepared by mixing 50 mL of 1.0 M sodium acetate solution with 39.5 mL of 1.0 M HCl solution and diluted to 250 mL with doubly distilled water. The pH of the solution was adjusted to an appropriate value with the aid of a pH meter.

Verapamil hydrochloride was kindly gifted by Samarth Pharma Pvt. Limited (Mumbai, India). The standard solutions of verapamil hydrochloride (2.5 mg mL^{-1} and 0.4 mg mL^{-1}) were prepared in doubly distilled water.

Preparation of Degraded Verapamil Hydrochloride

0.5 g Verapamil hydrochloride was treated with 5.0 mL of 1.0 M sodium hydroxide or hydrochloric acid at 70 ± 1°C for 72 h to produce its degraded product. The excess of sodium hydroxide or hydrochloric acid was neutralized with the corresponding volume of hydrochloric acid or sodium hydroxide and then diluted with water. Preparative thin layer chromatography was applied using silica gel G plate and ethyl acetate-methanol-chloroform (10:1.3: 1 v/v/v) as mobile phase for isolation of the degraded product. The band corresponding to the degradation product was located under UV lamp at 278 nm and was scraped and extracted with chloroform. The solvent was removed and pure degradation product was obtained.

Recommended Procedures
Method A

Into a series of boiling test tubes different concentrations of verapamil hydrochloride (0.05 - 0.75 mL; 0.25%) solution were pipetted. To each test tube 1.2 mL of 0.2 % potassium metaperiodate and 5 mL of 10 M H$_2$SO$_4$ were added, mixed well and placed on a water bath maintained at 100±1°C for 18 min. The

solutions were cooled to room temperature and transferred to a 10 mL standard volumetric flask. The contents were diluted to volume with doubly distilled water. The absorbance was measured at 425 nm within the stability period of 2 h against the reagent blank treated similarly.

Method B

Into a series of 50 mL separating funnels different volumes of 0.04% drug solution containing up to 300 mg were pipetted. 5 mL of buffer solution of pH 4 and 2 mL of 0.05 % tropaeolin 000 No.1 were added to each separating funnel and mixed well. The funnels were shaken with 10 mL chloroform for 2 min for complete extraction and then allowed the two layers to separate. The absorbance of the chloroform layer was measured at 400 nm against a reagent blank treated similarly. The color is stable for at least 3 h

Analysis of Pharmaceutical Formulations

Twenty tablets were powdered and mixed thoroughly. An amount equivalent to 250 mg of verapamil hydrochloride was weighed accurately and extracted with chloroform to eliminate any interference from excipients. It was filtered through Whatmann No. 42 filter paper and the residue was washed well with chloroform for complete recovery of the drug. The chloroform was evaporated to dryness and the drug was dissolved in doubly distilled water and diluted to 100 mL with doubly distilled water. It was further diluted if needed and then analyzed following the recommended procedures.

The whole content of ten ampoules (VPL; 5 mg (2 mL)$^{-1}$) equivalent to 50.0 mg (20 mL)$^{-1}$ of verapamil hydrochloride was taken to get the concentration of 2.5 mg mL^{-1} (0.25%). A 8.0 mL volume of 0.25% verapamil hydrocloride solution was diluted with doubly distilled water in 50.0 mL standard volumetric flask to get the test solution of 0.04 % verapamil hydrochloride. The two solutions were then subjected to the recommended procedures for the determination of verapamil hydrochloride.

RESULTS

Mechanism of the Color Reaction

Potassium metaperiodate is a well-known oxidant and has been used for the oxidation of β-blockers[18,19,20] containing amino group. The products of the oxidation were arylacetaldehde and secondary or tertiary butylamine. In all such studies, the presence of a methyl or methylene group attached to nitrogen is the basic need so that >N¾CH$_2$ linkage in tertiary amines was cleaved preferentially by an oxidant (potassium metaperiodate or chloramine T or N-

bromosuccinimide) resulting in the formation of yellow colored product. The oxidation of verapamil hydrochloride with potassium metaperiodate was carried out in aqueous sulfuric acid medium as it fulfills the basic need of having tertiary amine with >N-CH$_2$ linkage. Therefore, >N-CH$_2$ linkage was cleaved preferentially resulting in the formation of yellow colored product of arylacetaldehyde and secondary amine. The oxidation product absorbs maximally at 425 nm. In order to establish stoichiometric ratio between verapamil hydrochloride and potassium metaperiodate, the method of continuous variations[21] has been applied and was found to be 1:1. Therefore, based on the literature background[20] and our experimental findings, the reaction mechanism was proposed and given in Scheme 1.

The two major products were isolated; one produced a blue color when treated with a benzene solution of chloranil confirming the presence of amino group, and the other product reacted with 2,4-dinitrophenyl hydrazine to give yellow colored product indicating the presence of arylacetaldehyde. It has been confirmed that iodine was not evolved during this reaction.

Tropaeolin 000 No. 01 is chemically known as 4-(4-hydroxy-1-naphthylazo) benzene sulfonic acid sodium salt. It is a water soluble dye exhibiting absorption band peaking at 480 nm and producing –SO$_3^-$ group on dissociation. The amino group of verapamil hydrochloride is protonated and hence forms ion-pair complex with tropaeolin 000 No.1 which is quantitatively extracted from aqueous solution over the pH 3.5-4.5 into chloroform. The ion-pair complex absorbs maximally at 400 nm. The reagent blank under similar conditions showed no absorption at 400 nm because tropaeolon 000 No.1 is insoluble in chloroform. The molar ratio between verapamil and tropaeolin 000 No.1 was found to be 1:1 by Job's method. The reaction mechanism is given in Scheme 2.

Solution Stability

The stability of verapamil hydrochloride solution was investigated by recording absorption spectra of the solution for several days and by TLC studies. The band corresponding to degradation product was not observed under UV lamp at 278 nm, suggesting almost

(3,4-dimethoxyphenyl)acetaldehyde

5-(N-methyl amino) 2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitile
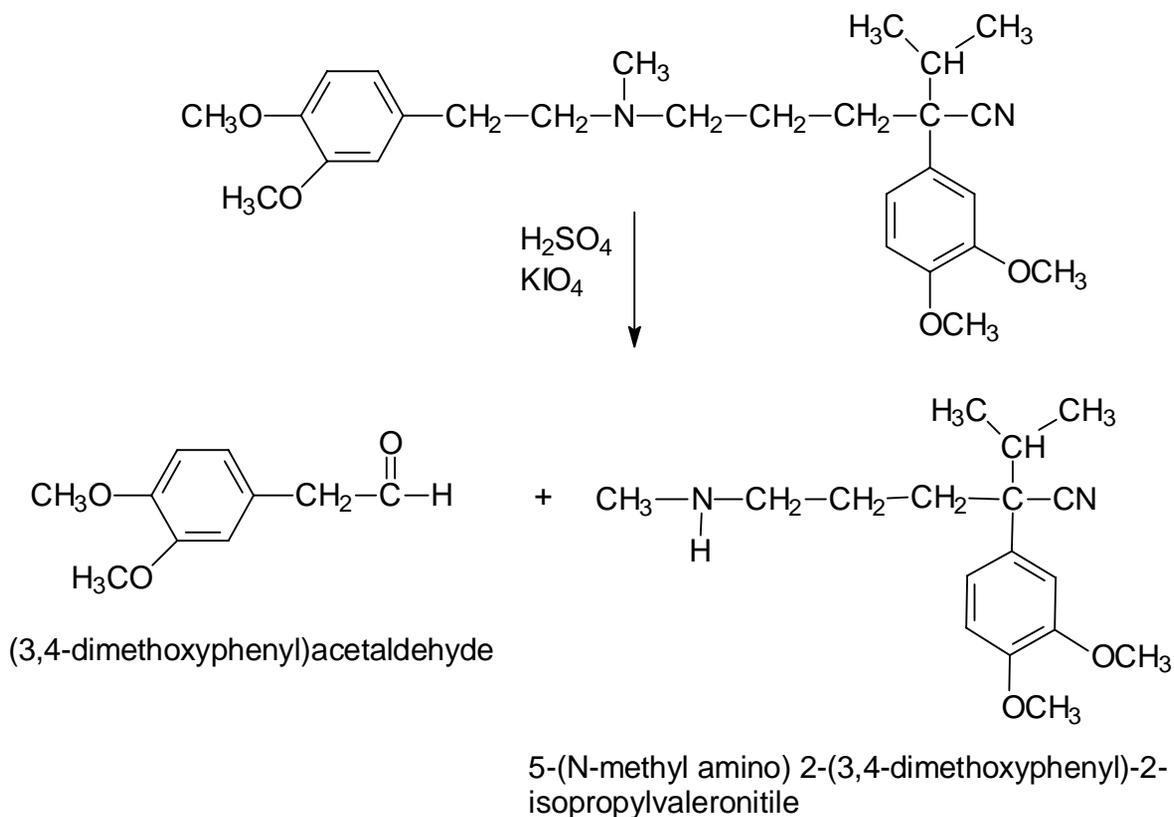
Scheme 1

no degradation. There was also no change in the spectra for at least four days, when the solution was stored at room temperature (25 ±1 °C).

Specificity

The specificity of the proposed methods was evaluated by determining the verapamil hydrochloride concentration in the presence of varying amounts of degraded verapamil. It was found that the degradation product did not interfere with the determination process.

Optimization of Variables

The optimum conditions for the assay procedures (methods A and B) have been established by studying the reactions as a function of heating time, concentration of reagents, solution stability and the stability of the colored species.

Method A
Effect of the Concentration of Sulfuric Acid

The influence of the volume of 10 M H_2SO_4 on the formation of yellow color was studied. This was performed by pipetting 0.75 mL of verapamil hydrochloride (0.25%), 1.5 mL of potassium metaperiodate and different volumes (1-5 mL) of 10 M H_2SO_4. The maximum absorbance was obtained with 4.7 mL of 10 M H_2SO_4. Above this volume, the absorbance remains constant. Therefore, 5.0 mL of 10 M H_2SO_4 was used for all the measurements.

Effect of Heating Time

To investigate the optimum heating time for color development, 0.75 mL of 0.25% verapamil hydrochloride was mixed with 5 mL of 10 M H_2SO_4 and 1.2 mL of 0.2% potassium metaperiodate into a boiling test tube. The content of the mixture was heated for 17-20 min on a water bath at 100±1°C. The maximum intensity of color was obtained at 17 min of heating and remained constant up to 20 min. Therefore, 18 min of heating time was used throughout the experiment.

Effect of Potassium Metaperiodate

To 0.75 mL of 0.25% verapamil hydrochloride, different volumes (0.5-1.5 mL) of 0.2% potassium metaperiodate and 5 mL of 10 M H_2SO_4 were added.

protonated verapamil

tropaeolin 000 No.1

Verapamil-tropaeolin 000 No.1 ion-pair complex
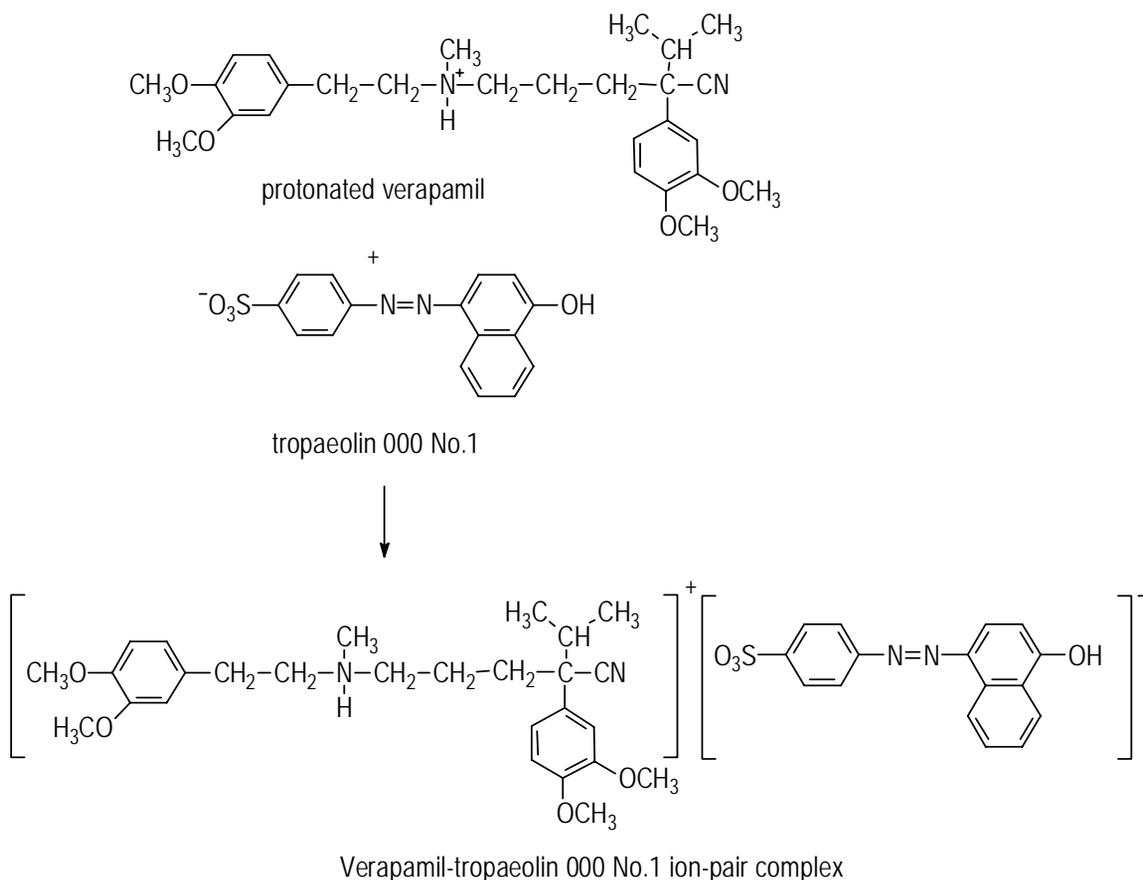
Scheme 2

Table 1. Standard addition method for the determination of verapamil hydrochloride in dosage forms.

Formulations	Method A						Method B					
	Amount (µg mL⁻¹)			Recovery (%)[a]	SAE[b]	C.L.[c]	Amount (µg mL⁻¹)			Recovery (%)[a]	SAE[b]	C.L.[c]
	Taken	Added	Found ± SD[a]	± RSD[a]			Taken	Added	Found ± SD[a]	± RSD[a]		
Tablets												
Calaptin 80	20.0	30.0	50.20 ± 0.37	100.40 ± 0.74	0.17	0.46	4.0	4.0	8.00 ± 0.04	100.01 ± 0.52	0.02	0.05
(Nicholas Piramal)	50.0	50.0	100.04 ± 0.35	100.04 ± 0.35	0.16	0.43	8.0	12.0	20.10 ± 0.21	100.49 ± 1.02	0.09	0.25
Verap 80	20.0	30.0	50.12 ± 0.23	100.24 ± 0.45	0.10	0.28	4.0	4.0	7.98 ± 0.04	99.76 ± 0.52	0.02	0.05
(Mano Pharm)	50.0	50.0	100.12 ± 0.48	100.12 ± 0.48	0.21	0.59	8.0	12.0	20.09 ± 0.20	100.44 ± 0.99	0.09	0.25
Injection												
VPL Amp 05/2 mL	20.0	30.0	50.04 ± 0.54	100.08 ± 1.09	0.24	0.67	4.0	4.0	8.02 ± 0.05	100.26 ± 0.56	0.02	0.06
(Samarth Pharma)	50.0	50.0	100.04 ± 0.35	100.04 ± 0.35	0.16	0.43	8.0	12.0	20.11 ± 0.19	100.54 ± 0.94	0.08	0.23

[a] Mean for five determinations.
[b] SAE, standard analytical error.
[c] C.L., confidence limit at 95% confidence level and four degrees of freedom (t = 2.776).

The reaction mixtures were heated for 18 min on a water bath at $100 \pm 1°C$ to develop maximum color. The absorbance was measured against a reagent blank at 425 nm. The highest absorbance was obtained with 1.0 mL, which remained unchanged with higher amount of potassium metaperiodate. Thus, 1.5 mL of the reagent was added for color measurements.

Method B
Effect of the pH
The influence of pH of the buffer solution of sodium acetate-HCl on the formation of extractable ion-pair complex of the drug with tropaeolin 000 No.1 was studied. It was observed that the absorbance of the complex with tropaeolin 000 No.1 was found to be constant within the pH range 3.5-4.5. Thus, all the absorbance measurements were made at pH 4.

Effect of the Concentration of Tropaeolin 000 No.1
The effect of the volume of 0.05% tropaeolin 000 No.1 was studied in a series of 50 mL separating funnels containing 30 µg mL⁻¹ of verapamil hydrochloride, 5.0 mL of buffer solution of pH 4 and different volumes (0.5-2.5 mL) of the reagent. 10 mL of chloroform was used to extract the ion-pair complex. The maximum absorbance was found with 1.9 mL of tropaeolin 000 No.1, beyond which absorbance was constant. Thus, 2.5 mL of tropaeolin 000 No.1 was used for ion-pair formation throughout the experiment.

Choice of Organic Solvent and Time of Shaking
A variety of organic solvents such as benzene, toluene, hexane, chloroform, carbon tetrachloride, ethyl acetate and 1,2-dichloromethane were examined for an applicable extraction procedure, but chloroform was preferred for its selective extraction of the ion-pair complex from the aqueous solution.
The time of shaking for complete extraction of ion-pair complex was studied and found that the absorbance of the extract remains constant between 0.5-4.0 min. Thus, 2.0 min shaking time was utilized as an optimum value throughout the experiment. The ion-pair complex was quantitatively recovered in one extraction only.

Accuracy and Precision
The five independent analyses of verapamil hydrochloride samples at three different concentration levels (25.0, 100.0 and 175.0 µg mL⁻¹ for method A and 4.0, 16.0 and 28.0 µg mL⁻¹ for method B) were performed. The relative standard deviations in intra day and inter day assays were found in the range 0.13-0.91 % and 0.25-1.20 % respectively, by method A and 0.20-1.04 % and 0.35-1.30 % respectively, by method B.
To check the validity of the proposed methods

Table 2. Point and interval hypothesis tests: comparison of the proposed methods with the reference method.

Formulations	Method A						Method B						Reference Method	
	Recovery (%)[a]	RSD (%)[a]	t-value[b]	F-value[b]	q_L^c	q_U^c	Recovery (%)[a]	RSD (%)[a]	t-value[b]	F-value[b]	q_L^c	q_U^c	Recovery (%)[a]	RSD (%)[a]
Tablets														
Calaptin 80 (Nicholas Piramal)	100.30	0.28	0.48	2.03	0.989	1.015	99.96	0.15	0.53	1.81	0.989	1.008	100.07	0.20
Verap 80 (Mano Pharma)	100.21	0.33	0.29	1.02	0.984	1.019	99.93	0.27	0.17	1.47	0.983	1.015	100.03	0.33
Injection														
VPL Amp 05/2 mL (Samarth Pharma)	100.30	0.28	0.48	20.26	0.989	1.015	99.96	0.25	0.25	1.59	0.987	1.011	100.07	0.20

[a] Mean for five determinations.
[b] Theoretical t-value (n = 8) and F-value (n = 4,4) at 95% confidence level are 2.306 and 6.39, respectively.
[c] In pharmaceutical analysis, a bias, based on recovery experiments, of ±2% (q_L=0.98 and q_U=1.02) is acceptable.

using standard addition technique, a fixed amount of pure drug was added to the preanalysed drug formulations. The results are summarized in Table 1. The recovery results were quite satisfactory.

Ruggedness

The method ruggedness was evaluated by a second analyst using a different instrument (A Shimadzu 1601 UV-visible spectrophotometer, Japan) and freshly prepared standard and sample solutions. The analysis of the verapamil tablets and injection was carried out five times at one concentration level by the ruggedness chemist and developing chemist following the recommended procedures. The results agreed well within the acceptable limits and no degradate was found to interfere with the determination process. These results demonstrated acceptable method ruggedness for routine quality control analysis of active drug in pharmaceutical formulations.

Robustness

The operational parameters of Methods A and B for the assay of verapamil hydrochloride tablets and injection were found to be very robust. The operational parameters investigated were:

For Method A
- volume of 10 M H_2SO_4 (± 0.3 mL).
- volume of 0.2% potassium metaperiodate (±0.5 mL).
- solution heating temperature (± 2° C).
- solution cooling temperature (± 4° C).
- solution heating time (± 1 min).

For Method B
- volume of 0.05% tropaeolin 000 No.1 (±0.5 mL).
- Influence of pH (± 0.5 pH).
- solution shaking time (± 0.5 min).

The robustness of the proposed methods relative to each operational parameter was examined by analyzing the verapamil tablets and injection under variable experimental conditions. For this, a sample solution of different drug formulations containing 30 µg mL⁻¹ of active drug was assayed five times using methods A and B (Table 2). The results showed good mean recovery with low relative standard deviation. Thus the proposed methods were found to be very robust for routine quality control analysis of drug in pharmaceutical formulations.

DISCUSSION

Analytical Data

Under the optimized experimental conditions, linear calibration graphs were obtained over the concentration ranges 12.5-187.5 and 2-30 µg mL⁻¹ of verapamil hydrochloride with molar absorptivities of 1.18×10^3 and 9.82×10^3 l mol⁻¹cm⁻¹ using methods A

and B, respectively. Table 3 summarizes the analytical parameters such as Beer's law limit, calibration equations obtained by least square treatment of calibration data, coefficient of correlation, confidence limit for slope and intercept, and variance. The correlation coefficient was found to be 0.9999 and 0.9998 for methods A and B, respectively, indicating the good linearity of both the calibration graphs and the intercepts are all close to zero. Test of significance of the intercept of the regression line showed that the experimental intercept, a, does not differ significantly from the theoretical value, zero. For this, a simplified method was used to calculate the quantity from the relation $t = a/S_a$ and its comparison with the tabulated data from the t-distribution. The t-value for methods A and B are 0.200 and 0.190, respectively, which do not exceed the tabulated t-value (t = 2.365, when n = 7) at 95% confidence level. It confirmed that intercepts for the proposed methods are not significantly different from zero. Thus the present methods are free from constant errors independent of the concentration of the verapamil hydrochloride.

The detection limit[22] and variance (S_0) were found to be 0.77 μg mL^{-1}, 7.01×10^{-7} μg mL^{-1} and 0.40 μg mL^{-1}, 1.28×10^{-5} μg mL^{-1} for methods A and B, respectively. The small value of variance obtained for both the methods pointed towards negligible scattering of experimental data points around the line of best fit.

The statistical analysis of the calibration data has been used to calculate error (S_c)[23] in the determination of a given concentration of verapamil hydrochloride and also provides a way to establish the confidence limits at the selected levels of confidence to estimate the relative uncertainty of the concentration over the full range of the concentrations tested. The error reached a minimum value when the measurement is made at 16 and 100 μg mL^{-1} for methods A and B, respectively.

Point and Interval Hypothesis Tests

The applicability of the proposed method was compared with the reference method[16] using point hypothesis tests by analyzing authentic commercial products of verapamil hydrochloride such as Calaptin 80 (Nicholas Piramal), Verap 80 (Mano Pharma) and VPL Amp. 05/2 mL (Samarth Pharma). Table 2 shows that the calculated t- and F- values are less than the theoretical ones, confirming accuracy and precision are within the acceptable limits and indicating no significant difference between the performance of the proposed methods and the reference method at 95% confidence level. The interval hypothesis tests[24] have also been performed to compare results of the proposed methods (A and B) with those of the reference method at 95% confidence level (Table 2). The Canadian Health Protection Branch has recommended that a bias, based on recovery experiments, of ± 2 % (q_L = 0.98 and q_U =1.02) is acceptable.[25] The results of the proposed methods were statistically compared with those obtained by the reference method. It is evident from Table 2 that the true bias of all samples is smaller than ± 2%. Once this is established, the actual error is ignored, and not reported along with the concentration itself. The interval hypothesis tests draw the same conclusion as the point hypothesis tests.

CONCLUSIONS

Method A is selective because verapamil hydrochloride can be determined in presence of its degraded product. It is superior to other existing spectrophotometric methods[13,14,15] as it provides a wide linear dynamic range of determination. However, the demerit of this method is that it requires heating for 18 min to develop the color. Method B does not involve any critical reaction conditions and tedious sample preparations. The range of determination is wider with low values of relative standard deviation as compared to other existing extractive spectrophotometric method.[16] The applicability of the proposed procedures for routine quality control is well established by assay of verapamil hydrochloride in bulk form and pharmaceutical preparations.

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