VISIBLE SPECTROPHOTOMETRIC METHODS FOR THE ESTIMATION OF MODAFINIL IN PHARMACEUTICAL FORMULATION

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ABSTRACT

Two simple, sensitive, and fast visible spectrophotometric techniques for quantifying modafinil in marketed dosage form have been proposed. The method A was focussed on the complexation involving charge transfer with picric acid to give pale yellow colored chromogen which shows absorption maxima at 450 nm. In the concentration range of 5-40 µg mL^{-1}, Beer's law is obeyed with a correlation value of 0.999. The method B depends on the oxidative coupling process with MBTH in the presence of $FeCl_3$ in acidic pH to give green colour chromogen which shows absorption maxima at 657 nm. In the concentration range of 10-50 µg mL^{-1}, Beer's law was obeyed with a correlation value of 0.999. The developed methodologies have been statistically verified for use in quality control laboratories for pharmaceuticals.

Keywords: Modafinil, visible spectrophotometric, picric acid, MBTH

INTRODUCTION

Modafinil (Fig. 1) is chemically 2- (diphenyl methane) sulfinylacetamide and is used for the treatment of narcolepsy. Narcolepsy is caused by the malfunctioning of the orexins, a family of peptides that promote alertness and prevent sleep, and whose neurons are stimulated by modafinil.

The chemical structure is represented by the formula $C_{15}H_{15}NO_2S$, and its molecular weight is given as 273.35 g mol^{-1}. It is not official in any of the pharmacopoeias but listed in Merck Index and Martindale[1], the complete drug reference. The reported methods for the quantification of modafinil in physiological samples are high performance liquid chromatography[2-6], gas chromatography–mass spectrometry[7] and liquid chromatography-mass spectrometry[8]. Few HPLC[9-12] and HPTLC[13] techniques have been referenced for the quantification of modafinil in bulk and marketed forms. The above reported chromatographic methods employed are sophisticated and expensive. There is only one UV[14] and a few visible spectrophotometric[15-16] methods published for estimation of modafinil in marketed formulation. In the current work, two simple visible spectrophotometric techniques for the analysis of modafinil with acceptable linearity, precision and accuracy have been established and validated. These approaches could be utilized in the quantification of modafinil in their marketed form.

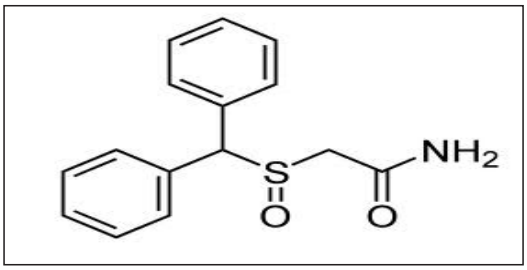

Fig. 1: Structure of modafinil

MATERIALS AND METHODS

Instrumentation and reagents

Using an ELICO-SL 210 UV-Visible double beam spectrophotometer, spectral and absorbance measurements were taken with spectral bandwidth 1.8 nm and accuracy of ±0.5 nm in spectrum mode and accuracy of ± 0.005 Abs at 1.0 Abs in photometric mode. Paired 10 mm quartz cells were employed for spectral measurement. The system was equipped with Spectral Treats software. Tablet formulation MODALERT-100 (Modafinil Tablets) containing modafinil 100 mg was chosen in the proposed investigation. Picric acid and MBTH were from Sigma Aldrich; Ferric chloride, dichloromethane, methanol and HCl from S.D. Fine Chemicals, Mumbai and double distilled water was analytically pure.

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Preparation of reagents

2 % w/V Picric acid solution: 2 g of picric acid was dissolved in 100 mL of dichloromethane.

0.5 % w/V 3-methyl-2-benzthiazolinone hydrochloride (MBTH): Accurately weighed, 0.5 g of MBTH reagent was put into a 100 mL volumetric flask, solublized and was brought up to the level with double distilled water.

2 % w/V $FeCl_3$ (w/V): 2 g of ferric chloride was mixed with 100 mL of 0.1N HCl.

0.1N HCl: Prepared by adding 100 mL of double distilled water to 0.85 mL of conc. HCl.

Principle for method A

In method A, modafinil, an n-donor (D), forms a pale yellow coloured charge-transfer complex (C-T) with picric acid(A) ϖ acceptor in dichloromethane and the colored species that resulted had a maximum absorbance of 450 nm. This colour changed into pale yellow when the picric acid was mixed with the drug, and showed an absorption peak at 450 nm . The protonation of the $-NH_2$ group in modafinil happens by a proton-transfer phenomenon from the acidic site of picric acid acceptor to nitrogen's lone pair of electrons in modafinil based on acid-base theory as acceptor to produce charge transfer complexes as shown in Scheme 1.

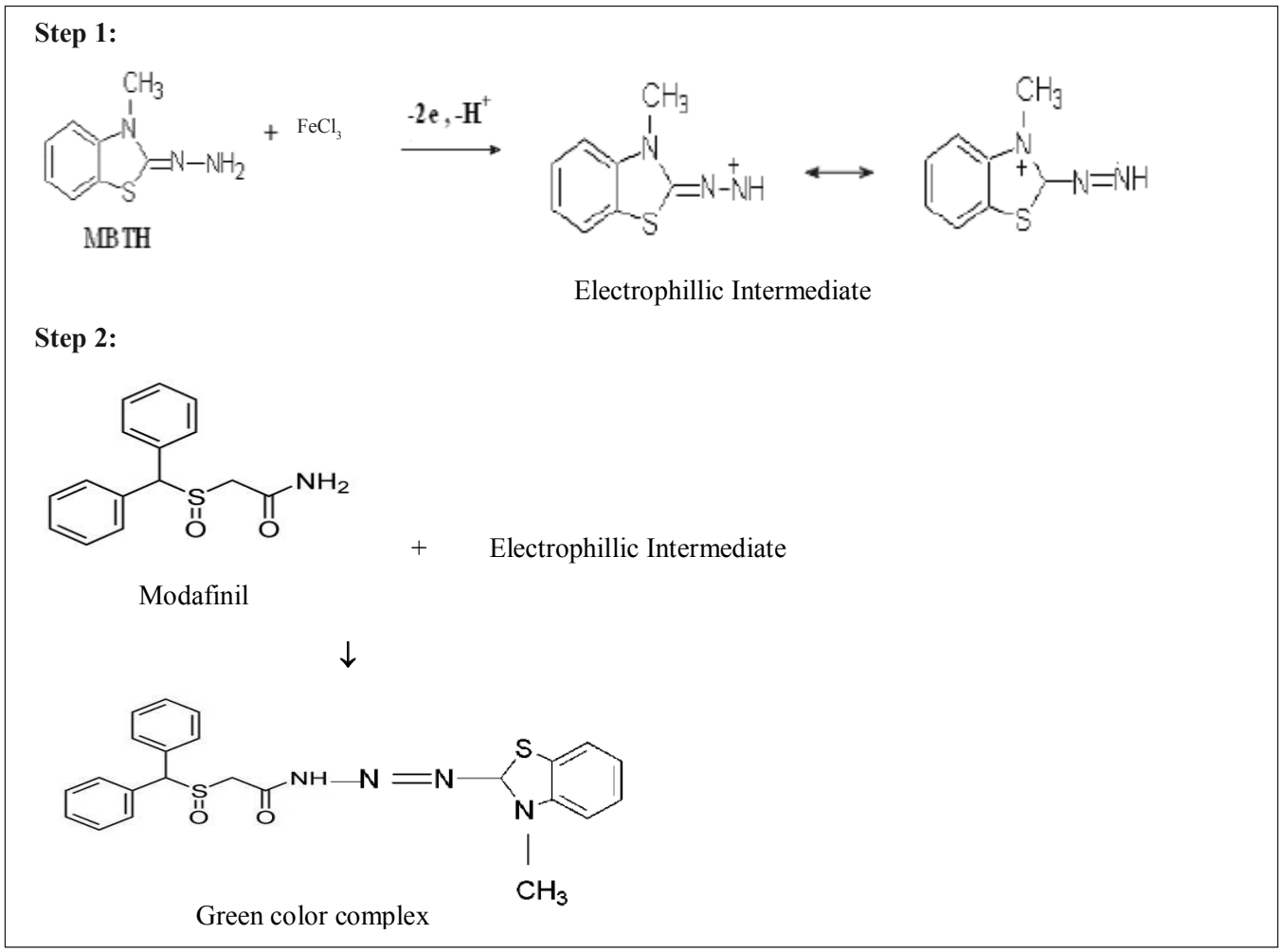
$$D^{\cdot} + A \rightleftharpoons \underset{\text{Complex}}{(D\text{-}A)} \rightleftharpoons \underset{\text{radical ions}}{D^{\cdot+} + A^{\cdot-}}$$

Scheme 1: Reaction pathway for the formation of the electron donor–acceptor complex and radical ions between modafinil and picric acid

A= π Acceptor , D=Donor

Principle for method B

Method B produces a green product with an absorbance peak at 657 nm when modafinil combines



Scheme 2: Reaction of MBTH with modafinil

with MBTH in an acidic condition containing $FeCl_3$. Actually, the drug and MBTH are oxidatively coupling in a process that is iron catalyzed. The active coupling species is created according to reaction conditions in which the oxidation of MBTH results in the loss of two electron and one proton, producing an electrophilic intermediate. With the drug, this intermediate undergoes electrophilic substitution to produce the chromatic compound. The hypothetical reaction scheme is provided (Scheme 2).

Preparation of standard stock solution (100 µg mL^{-1}) --- (Methods A & B):

Weighed accurately about 10 mg of modafinil and transferred it into a 100 mL volumetric flask. The content of the flask was dissolved with little quantity of methanol and volume was made up to the mark with same solvent.

Procedure for calibration curve

Method A

Aliquots of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mL of 100 µg mL^{-1} modafinil standard solution were accurately transferred into a series of 10 mL calibrated flasks and to these solutions, 1 mL of 2 % w/V of picric acid solution were added to each flask, the content was mixed well and the flasks were allowed to stand for 10 min. The volume was brought to the mark with dichloromethane. A pale yellow colour was developed and the absorbance of each solution was measured at 450 nm against the reagent blank. The absorbance spectrum is shown in Fig 2. A calibration graph for modafinil was plotted by taking concentration of drug on x-axis and absorbance on y-axis.

Method B

Aliquots of 1.0, 2.0, 3.0, 4.0 and 5.0 mL of 100 µg mL^{-1} modafinil standard solution were accurately transferred into a series of 10 mL calibrated flasks and to these 1 mL of MBTH and 1 mL of 2 % w/V ferric chloride were added to each flask and the volume was brought to the mark with double distilled water and allowed to stand for 20 min. A green colour was developed and the absorbance of each was measured at 657 nm against the reagent blank. The absorbance spectrum is shown in Fig. 3. A calibration graph for modafinil was plotted by taking concentration of drug on x-axis and absorbance on y-axis.

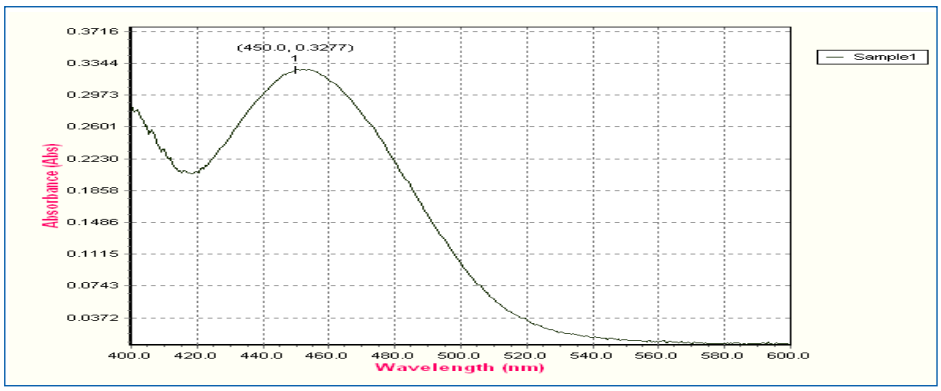


Fig. 2: Visible spectra of modafinil with picric acid

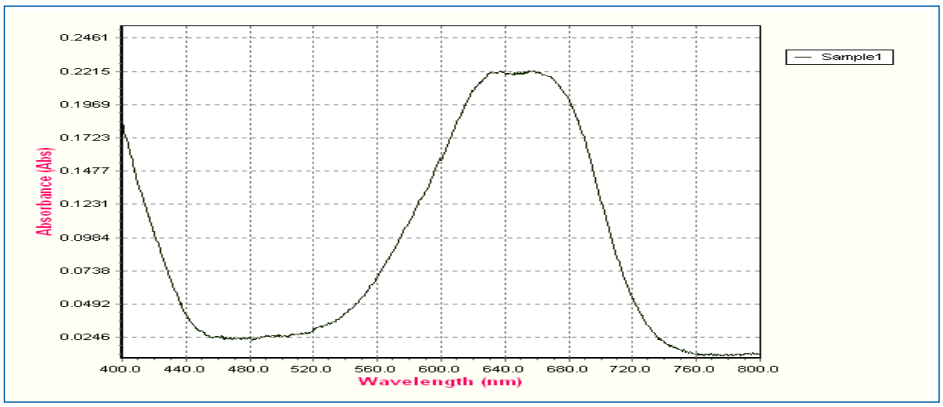


Fig. 3: Visible spectra of modafinil with MBTH

Estimation of modafinil in tablet formulation for method A

Marketed tablet formulations containing 100 mg of modafinil were analyzed by this method. From the triturate of 20 tablets, an amount equivalent to 100 mg of modafinil was weighed and transferred to a 100 mL volumetric flask. The contents of the flask were dissolved in about 40 mL methanol. The solution was filtered through Whatman filter paper no. 41. The filter paper was washed with methanol. The washings were added to the filtrate and the final volume was made up to 100 mL with the methanol to get a concentration of 100 µg mL^{-1}. From the above solution 2 mL was transferred to 10 mL calibrated volumetric flask and to this solution 1 mL of 2 % w/V picric acid solution was added, the contents were mixed well and the flask was allowed to stand for

10 min. The volume was brought to the mark with dichloromethane. A pale yellow colour was developed and the absorbance of the final sample corresponding to 20 µg mL^{-1} was measured at 450 nm against the reagent blank. The amount of drug was calculated from calibration curve. The results of analysis of tablet formulations are recorded in Table I.

Estimation of modafinil in tablet formulation for method B

Marketed tablet formulations containing 100 mg of modafinil were analyzed by this method. From the triturate of 20 tablets, an amount equivalent to 100 mg of modafinil was weighed and transferred to 100 mL volumetric flask. The contents of the flask were dissolved in about 40 mL methanol. The solution was filtered through Whatman filter paper no. 41. The filter paper was washed with methanol. The washings were added to the filtrate and the final volume was made up to 100 mL with methanol to get a concentration of 100 µg mL^{-1}. From the above solution, 2 mL was transferred to 10 mL calibrated volumetric flask and to this 1 mL of MBTH, 1 mL of 2 % w/V ferric chloride were added, the contents were mixed well and the flask was allowed to stand for 20 min. The volume was brought to the mark with double distilled water. The green colour was developed and the absorbance of the final sample corresponding to 20 µg mL^{-1} was measured at 657 nm against the reagent blank. The amount drug was calculated from calibration curve. The results of analysis of tablet formulations are recorded in Table I.

Table I: Estimation of modafinil in tablets

Methods	Assay sample	Labeled amount (mg)	Amount found* (mg)	%Purity ± SD*
Method A	MODALERT-100	100 mg	99.78 mg	99.78% ± 0.02
Method B	MODALERT-100	100 mg	99.45 mg	99.45 % ±0.049

** Average of three determinations*

RESULTS

Validation of analytical data[17]

The method was validated in accordance with the current ICH guidelines.

Linearity and range

The calibration graphs obtained by plotting the values of the absorbance versus the concentrations (µg mL^{-1})

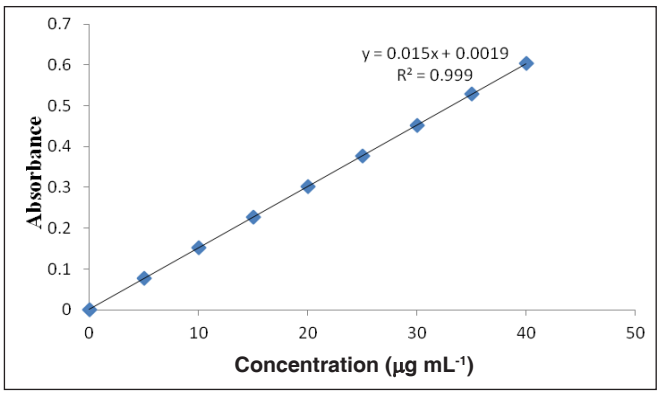


Fig. 4: Beer's law plot of modafinil with picric acid

were found to be linear over the concentration range of 5-40 µg mL^{-1} and 10-40 µg mL^{-1} for methods A and B, respectively. From the data obtained, co-relation coefficient, slope and y-intercept were calculated and the results are recorded in Table II. Ideally co-relation

Table II: Optical characteristics of modafinil

S. No.	Parameter	Method A	Method B
1.	Absorption maxima (nm)	450	657
2.	Linearity range (µg mL^{-1})	5-40	10-50
3.	Regression equation	y = 0.015x + 0.003	y = 0.003x - 0.002
4.	Slope (b)	0.015	0.003
5.	Intercept (a)	0.003	- 0.002
6.	Correlation coefficient (r^2)	0.999	0.999
7.	%RSD	0.32	0.38
8.	Molar absorptivity (L mol^{-1} cm^{-1})	4.3×10^3	1.2×10^3
9.	Sandell's sensitivity (µg cm^{-2}/0.001 abs unit)	0.063	0.225
10.	% Range of errors (Confidence limits)		
	0.01 %	0.614	0.644
	0.05 %	0.945	0.915
11.	Limit of detection (µg mL^{-1})	0.185	0.187
12.	Limit of quantification (µg mL^{-1})	0.534	0.543

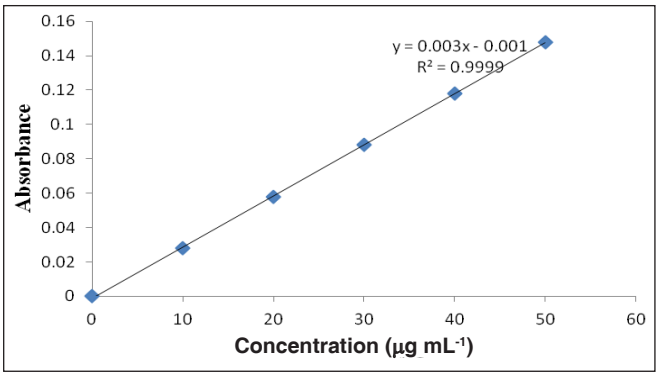


Fig. 5: Beer's law plot of modafinil with MBTH

coefficient should be not less than 0.998. Beer's plots are shown in Fig. 4 & 5.

Precision

The precision of analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. The system precision was determined by analysing six different solutions of same concentration and the absorbances were noted. The result are indicated by % RSD and shown in Table II. Repeatability or Intra-

Table III: Intraday and Interday precision for method A

	Intraday		Interday	
Conc. taken (µg mL^{-1})	Conc. found* (µg mL^{-1})	%RSD	Conc. found* (µg mL^{-1})	% RSD
10	9.84	0.36	9.89	0.38
20	19.92	0.61	19.91	0.55
30	29.86	0.38	29.85	0.35

**Average of six determinations*

Table IV: Intraday and Interday precision for method B

	Intraday		Interday	
Conc. taken (µg mL^{-1})	Conc. found* (µg mL^{-1})	%RSD	Conc. found* (µg mL^{-1})	%RSD
20	19.98	0.34	19.95	0.38
30	29.93	0.36	29.96	0.21
40	39.95	0.36	39.14	0.45

**Average of six determinations*

Table V: Accuracy of method A

S. No	% Level of recovery	Initial amount present (µg mL^{-1})**	Amount of standard added (µg mL^{-1})**	Total amount present (µg mL^{-1})**	Standard amount recovered (µg mL^{-1})**	% Recovery ±SD**
1.	80	20	16	36	15.92	99.37 ± 0.281
2.	100	20	20	40	19.88	99.4 ± 0.312
3.	120	20	24	44	23.94	99.75 ± 0.318

*** Average of three determinations*

Table VI: Accuracy of method B

S. No	%Level of recovery	Initial amount present (µg mL^{-1})**	Amount of standard added (µg mL^{-1})**	Total amount present (µg mL^{-1})**	Total amount recovered (µg mL^{-1})**	% Recovery ± S.D**
1.	80	20	16	36	15.82	98.87 ± 0.289
2.	100	20	20	40	19.78	98.90 ± 0.309
3.	120	20	24	44	23.83	99.29 ± 0.312

*** Average of three determinations*

day precision was investigated on six replicate sample solutions on the same day. Inter-day precision was assessed by analyzing newly prepared sample solutions in triplicate over three consecutive days. Both inter day and intraday precision are expressed as % RSD. The results are summarized in Table III & IV. The low value of % RSD for both methods indicates the high precision of the both methods.

Accuracy

Accuracy of the methods was determined by preparing solutions of different concentrations, that is, 80 %, 100 % and 120 % of targeted drug concentration in which the amount of marketed formulation was kept constant and the amount of pure drug was varied. Solutions were prepared in triplicate and accuracy was indicated by % recovery. The results are shown in Tables V & VI.

Robustness

The robustness of a method is its capacity to remain unaffected by small changes in conditions. To determine the robustness of the method, the experimental conditions were deliberately altered and assay was evaluated. The effect of detection wavelength was studied at ±2 nm. For changes of conditions, the samples were assayed in triplicate (Table VII and Table VIII).

Table VII: Results of robustness study (method A)

Formulation	Amount of drug taken from tablet (mg)	At 448 nm	At 452 nm
		(n=3) % Assay ± %RSD	(n=3) % Assay± % RSD
MODALERT-100	100	99.73± 0.313	99.91± 0.224

Table VIII: Results of robustness study (method B)

Formulation	Amount of drug taken from tablet (mg)	At 655 nm	At 659 nm
		(n=3)% assay ± %RSD	(n=3)% assay ± %RSD
MODALERT-100	100	99.61± 0.423	99.84± 0.516

Ruggedness

Ruggedness of the proposed method was determined by analysis of aliquots from homogeneous slot by two analysts using same operational and environmental conditions (Table IX and Table X).

Table IX: Ruggedness results of method A

S. No.	Labelled amount (mg)	Analyst 1		Analyst 2	
		Amount found (mg)	% Recovered ±S.D.**	Amount found (mg)	% Recovered ±S.D.**
1.	100 mg	99.96 mg	99.96 ± 0.022	99.88 mg	99.88± 0.042

*** Average of six determinations*

Table X: Ruggedness results of method B

S. No.	Labelled amount (mg)	Analyst 1		Analyst 2	
		Amount found (mg)	% Recovered ±S.D.**	Amount found (mg)	% Recovered ±S.D.**
1.	100 mg	99.20 mg	99.20 ± 0.033	99.82 mg	99.82± 0.041

*** Average of six determinations*

Limit of detection and Limit of quantitation

Limit of detection (LOD) and limit of quantitation (LOQ) were determined by using the formula based on standard deviation of the response and the slope. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated by using the equations LOD = 3 σ /S and LOQ= 10 σ /S, where σ is standard deviation of intercept, S is slope of the line (Table II).

DISCUSSION

The goal of the current research was to provide accessible, precise and sensitive visual spectrophotometric techniques for measuring the amount of modafinil in tablets. In Method A, modafinil, an *n*-donor (D), combines with picric acid, an acceptor, to generate a light yellow colored charge-transfer complex (C-T) in dichloromethane. The resultant colored species was found to absorb most effectively at 450 nm. By further refining the different reaction parameters, the process was optimized. On the absorbance, the impact of picric acid concentration was investigated. At 2 % w/V picric acid concentration, the maximum absorbance was attained. By permitting the reaction to continue for various time intervals, the impact of reaction time on the reaction was examined. It was noted that the reaction was completed in less than 10 min, indicating that prolonged response times did not significantly affect the reaction. So, 10 min reaction time was employed. Dichloromethane was utilized to dilute the reaction product since there were no solubility issues when utilizing it as the solvent. The permanence

of the color created under ideal circumstances was investigated by measuring the intensity of its absorption over time. The approach was shown to be acceptable for the examination of several samples since the colored complex's absorbance remained steady for two hours. The proposed visible spectrophotometric approach is simple when compared to the published visible spectrophotometric methods.

In technique B, modafinil interacts with MBTH in an acidic solution containing $FeCl_3$ to produce a green product with an absorbance peak at 657 nm. Actually, MBTH and the drug are oxidatively coupling in this process, which is mediated by iron. MBTH loses two electrons and a proton when it is oxidized under the reaction conditions, generating an electrophilic intermediate that couples. The coloured product is created when this intermediate and modafinil proceeds electrophilic substitution process. By further refining the different reaction parameters, the process was improved. Study was done on how MBTH concentration affected absorbance. The reaction product's absorbance increased together with the concentration of MBTH up to a certain level. At 0.5 % w/V, the maximum absorbance was obtained. As maximal absorbance was attained at this concentration, the ferric chloride concentration was tuned at 2 % w/V. By permitting the reaction to continue for various time intervals, the impact of reaction time on the reaction was examined. It was observed that the reaction was completed in less than 20 min, and that reaction periods greater than 20 min did not significantly affect the response. So, 20 min reaction time was employed. The permanence of the color created under ideal circumstances was investigated by measuring the intensity of its absorption over time. It was discovered that the colored complex's absorbance stayed constant for 2.5 h. Since water is both affordable and has no solubility issues with reaction products, it was utilized as a diluent. The proposed approach is sensitive when measured against the available visible spectrophotometric techniques. Optical characteristics like Beer's law limit, Sandell's sensitivity, molar absorptivity, percent relative standard deviation, and regression characteristics like slope, intercept, and percent range of error (0.05 and 0.01 confidence limits) were calculated and are presented in Table II. The visual spectrophotometric techniques created for modafinil were found to be simple, precise, economical, and repeatable. For methods A and B, the drug concentrations were found to be linear in the range of 5 to 40 µg mL^{-1} and 10 to 50 µg mL^{-1}, respectively. The linearity of the developed approaches is indicated by the correlation coefficient values of 0.999 for each. The fact that the intraday and interday percent relative standard deviation (percent RSD) for system precision were both 1 percent shows that the established procedures are precise. The excellent accuracy of the procedure is demonstrated by the percentage recovery figures for methods A and B, respectively, of 98.8 to 99.9 % and 99.8 to 100.1 %. The components often found in modafinil pharmaceutical formulations have no negative effects on the suggested procedures. The suggested approaches may thus be used to the routine assessment of pharmaceutical formulations based on the demands of a specific situation since they are simple, accurate, precise, and sensitive.

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