REPUBLIC OF TURKEY
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A NOVEL SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF MODAFINIL IN PHARMACEUTICAL FORMULATIONS USING QUINALIZARIN

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Master Thesis
Department: Chemistry
Supervisor: Prof. Dr. Habibe OZMEN

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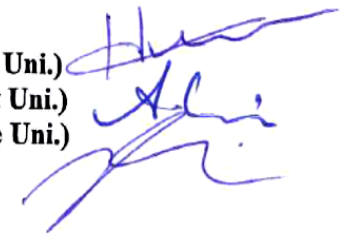

July 2017

DECLARATION

I declare that the Master Thesis entitled **"A Novel Spectrophotometric Method for Determination of Modafinil in Pharmaceutical Formulations Using Quinalizarin"** is my own research and prepared by myself, and hereby certify that unless stated, all work contained within this thesis is my own independent research and It is being submitted for the Degree of Master of Science (in analytical chemistry) at the Firat University, and has not been submitted for the award of any other degree at any institution.

DEDICATION

This thesis is dedicated to my beloved family, and I would like to thank them for their understanding, moral supports, encouragements, prayers, patience and all kind of support. It is also dedicated to my faithful friends.

ACKNOWLEDGEMENT

First of all, I would like to express my deep thanks to merciful **Allah**, who enabled me to perform and complete my scientific project successfully.

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I appreciate the role of Firat University and Faculty of Natural and Applied Science, Chemistry Department for giving me this great chance to study and got a certificate that will never be forgotten. Hope you all the best and delight.

I would like to thank GENERICA ILAC SAN VE TIC A.S for providing the pure modafinil drug

Finally, I would like to express my thanks to my friends for continuing to be a source of inspiration, and for all those precious moments which gave me a sense of direction.

Sincerely

BASHEER ISSA

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LIST OF CONTENTS

ABSTRACT

Simple, extraction free visible spectrophotometric method was developed and validated for the quantitative estimation of modafinil in pharmaceutical formulations and bulk drug using quinalizarin as analytical reagent**.** The method involve the reaction of modafinil with quinalizarin in alcoholic medium at room conditions to form a blue colored product exhibiting maximum absorption at 572 nm. Different variables affecting the reaction were studied and optimized. Under the optimized experimental conditions, Beer's law is obeyed in the concentration ranges of 20-180 $\mu g.mL^{-1}$ with the detection of limit value of 0.258 $\mu g.mL^{-1}$ for the method .The molar absorptivity for the method was reported. The method was validated in terms of accuracy, precision and robustness,the results were satisfactory. The proposed method was effectively applied to the analysis of the modafinil in their tablet formulations. Recovery studies for modafinil was found to be 99.9% . The %RSD value was found to be 0.6% for the method. The assay was not interfered by common excipients.

Keywords: Charge Transfer Reaction, Modafinil, Quinalizarin, Spectrophotometry, Pharmaceutical Formulations

ÖZET

Quinalizarin Kullanılarak Farmasötik Formülasyonlarda Modafinilin Tayini İçin Yeni Bir Spektrofotometrik Metod

Analitik reaktif olarak Quinalizarin kullanılarak farmasötik formülasyonlar ve ilaçlarda modafinilin kantitatif tayini için basit ve ekstraksiyonsuz spektrofotometrik metod geliştirilmiş ve doğrulanmıştır. Quinalizarin ile modafinilin reaksiyonu metilalkol ortamında oda şartlarında 572 nm dalgaboyunda maksimum absorbans veren yük transfer kompleksi olan mavi renkli ürün oluşturulmuştur. Reaksiyonu etkileyecek farklı değişkenler optimize edilmiş ve çalışılmıştır. Optimize edilen deneysel şartlar altında Beer's kanunu 20-180 $\mu g.mL^{-1}$ konsantrasyon aralığında, metod için tayin sınırı 0.258 $\mu g.mL^{-1}$elde edilmiştir. Molar absorptivite metod için belirlenmiştir. Metod doğruluk hassaslık ve sağlamlık açısından doğrulanmıştır. Sonuçlar tatmin edicidir. Önerilen metod tablet formülasyonlarında bulunan modafinilin analizi için etkili bir şekilde uygulanabilir. Modafinilin geri kazanımı 99.9% olarak bulunmuştur. %RSD değeri bu medod için 0.6% değeri elde edilmiştir. Denemelerde tabletlerde bulunan diğer maddeler girişim yapmamıştır.

Anahtar Kelimeler: Yük aktarım kompleksi, Modafinil, Quinalizarin, Spektrophotometri, Farmasötik formülasyon

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

1.1. Modafinil

Modafinil (MOD) belongs to the class narcoleptics. The chemical name is 2-[(Diphenylmethyl)-sulfinyl] acetamide (Figure 1.1). This is a1-adrenergic agonist. Clinical evaluation in hypersomnia and narcolepsy. It is not official in any of the pharmacopoeia. It is listed in the Merck Index 13th edition [1], and Martindale the complete drug reference 36th edition [2].

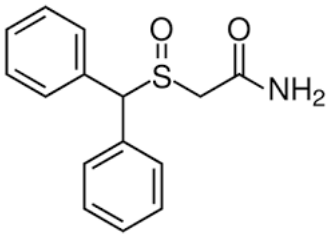

Figure 1.1.Chemical structure of modafinil

It was discovered in 1994 by the Laboratoire L. Lafon at a French pharmaceutical company. Modafinil is marketed as Provigil® by Cephlon Inc who originally leased the rights from Lafon and finally purchased the company in 2001[3, 4, 5]. Because of its wakening properties, absence of tolerance-producing effects and low toxicity, it could be used by armed forces in continuous or sustained processes including total or partial sleep deprivation. In 1998 it was been confirmed by the Food and Drug Administration (FDA) for treatment of narcolepsy and in 2003 for obstructive sleep apnea/hypopnea and shift work sleep disorders [6].

In recent times modafinil, a novel wake promoting drug has gained immense popularity among psychiatrists. Now a days, its use is not limited to treat sleep wake disorders and other narcolepsy, but also in attention deficit hyperactivity disorder, treatment resistant depression, chronic fatigue syndrome, and cocaine dependency[7]. Moreover, modafinil is increasingly being diverted for nonmedical use by healthy individuals with the expectation

that it will improve cognitive performance [8]. In addition, some academic doping has emerged because modafinil may enhance memory and attention [9] .

The exact action mechanism of modafinil is not known. By binding to the dopamine transporter, modafinil reduces the dopamine uptake and increases dopamine levels in the brain. It is pharmacologically and clinically different from other central nervous system stimulants in that it produces long lasting waking effects without, addictive attributes, sleep rebound or behavioral modification. It mimics the impact of amphetamines by producing a very high quality of wakefulness, but without some of the common side effects associated with amphetamine-like stimulants[10].

1.1.2. Physical–Chemical Properties of Modafinil

Modafinil is widely as wakefulness promoting agent for oral direction used. Its IUPAC name is [2-(1,1-diphenyl methyl sulfinyl) acetamide]. Its empirical formula and molecular weight is $C_{15}H_{15}NO_2S$, 273.35 daltons, respectively. Modafinil sparingly soluble in methanol, slightly soluble in ethanol and it is insoluble or slightly soluble in water. Its melting point is 160–165 ºC. According to Bio pharmaceutical classified system it is a white to off-white, crystalline powder (figure 1.2). It belongs to a class of drugs known as diphenylmethanes, which are stimulants that supply long- permanent mental arousal [11] .

Figure 1.2. Powder modafinil

1.2.Quinalizarin (Reagent)

Quinalizarin (1,2,5,8-tetrahydroxyanthraquinone) is a polyphenolic compound with formula $C_{14}H_8O_6$ (Figure 1.3). Originally used in the industry of pigments and dyes. It is one of many tetrahydroxyanthraquinone isomers, formally derived from anthraquinone by replacement of four hydrogen atoms by hydroxyl (OH) groups.

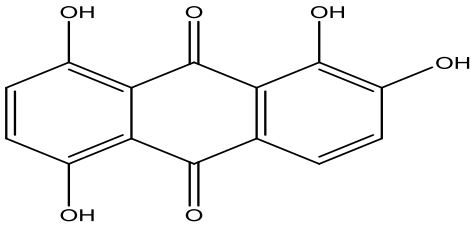

Figure 1.3. Chemical structure of quinalizarin

It has been measured a pollutant in waste waters from many texture industries since it is nonbiodegradable and very toxic to aquatic organisms. Quinalizarin is one of many tetrahydroxyanthraquinone isomers, presenting an asymmetric chemical structure responsible for peculiar chemical properties. It works as an acid-base indicator being orange in neutral/acidic solution, blue in mild base, and purple in strong base, thus presenting the deprotonation of one or two hydroxyl groups, respectively [12].Because of the quinalizarin chromogenic property make it good reagent for determination of different metal ions concentrations thanks to its ability to produce colored chelates. Many instances of this application have been reported since the early 1950s, for the detection of boron [13], uranium, molybdenum [14], and aluminium [15]. nowaday, a spectrophotometric method, based on quinalizarin complexation reaction, for estimation of thallium and manganese in biological samples and water has been applied [16,17]. A comparable method has also been accomplished to achieve the determination of two antiepileptics (pregabalin and gabapentin) in pharmaceutical tablets [18]. Furthermore, quinalizarin in cancer research has been used, being effective in various kinds of tumor cells (breast cancer) [19], prostate cancer [20], and leukemia T cells [21] and angiogenesis [22].]. It has been suggested as a promising drug prototype against human ganciclovir sensitive and ganciclovir-resistant cytomegalovirus [23],

and reported to inhibit growth of HIV on human peripheral blood mononuclear cells [24, 25]. In 2009 quinalizarin has been identified as a potent and selective inhibitor of protein kinase CK2 through a computer aided virtual screening and biochemical evaluation [21] and demonstrated to be a cell permeable compound able to inhibit endogenous CK2 in HEK-293 and Jurkat cells at a concentration<5 μM [21].

1.3. Charge Transfer Complex

The charge transfer (CT) or proton transfer (PT) complexes are formed from aweak interaction between an electron donor that has a sufficiently low ionization potential and an acceptor that has a sufficiently high electron affinity [26]. The formation of such complex is often characterized by the appearance of an intense, broad electronic absorption band in the UV– visible region that can be used for identification of the complex [27]. But, such band in the absorption spectra of both the donor and the acceptor species does not appears. The CT or PT interactions have attracted considerable interest due to their significant chemical and physical properties [28–29].

The charge transfer complex (CTC) term firstly was introduced by Mulliken [30-31], and discussed broadly by Foster [32]. Mulliken aimed to define a new type of adduct to explain the behavior of certain classes of molecules, which do not conform to classical patterns of ionic, covalent, and coordination of hydrogen bonding components. While such adducts largely retain some of the propertied of the components, some changes are apparent, e.g. its solubility, the diamagnetic and paramagnetic susceptibility. Other differences have also been found with electrochemical techniques. Some complexes can be isolated as crystals of regular stoichiometry and structure. One feature of charge transfer complexes is that the association constant of the complex decreases with increasing temperature. The effect is due to the thermal motion disorienting the partners of the complex. Mulliken [33,34] also showed that the charge transfer interactions within a molecular complex consisting of an electron donor D and an electron acceptor A involved a resonance with a transfer of charge from D to A:

$$D + A \leftrightarrow D^{+} A^{-} \leftrightarrow D^{+} + A^{-}$$

Indeed the charge transfer complexation achieved the great importance in biochemical, bioelectrochemical energy transfer methods [35], biological systems [36], and drug-receptor binding mechanism, for instances, enzyme catalysis, drug action, ion transfers through lipophilic membranes [37], and certain π-acceptors have successfully been utilized in pharmaceutical analysis of some drugs in pharmaceutical preparations and in pure form [38,39]. Latterly, many studies have been widely reported about the rapid interactions between different kinds of drugs and related compounds as donors like, norfloxacin, ciprofloxacin, sulfadoxine and, morpholine, with several types of n-electron and π-electron acceptors [40, 41]. On the other hand, electron donor-acceptor (EDA) interaction has a worth concern for chemical reactions like condensation, addition and substitution [42,43]. It displays an enormous important in many application fields and topics, like electrical conductivities and in non-liner optical materials [43-44], second order non-liner optical activity [46], micro emulsion [47], surface chemistry [47], photo catalysts [48], dendrimers [49], solar energy storage [50], organic semiconductors [51], as well as in studying redox processes [52]. Because of their special type of interaction the charge transfer complexes that using organic species are intensively studied, which is accompanied by the transfer of an electron from the donor to the acceptor [53, 54]. In addition, protonation of the donor from acidic acceptors is generally a way for the ion pair adducts formation [55, 56].

1.4. Selecting an Analytical Method

A method is the application of a technique to a specific analyte in a specific matrix. To select an analytical method intelligently, it is essential to define clearly the nature of the analytical problem. Such a definition requires answers to the following questions:

- What accuracy is required?
- How much sample is available?
- What is the concentration range of the analyte?
- What components of the sample might cause interference?

1.4.1. Validation Parameters

1.4.2. Specificity

The specificity describes the ability of the method to measure unequivocally the analyte of interest in the presence of all other components as interfering analytes [57].

1.4.3. Linearity

The relationship between the concentration of the analyte in the matrix and measured response has to be founded. Whenever possible, a suitable standard should be used for the quality control of the calibration lines.

1.4.4. Accuracy

Accuracy is a measure of how narrowly the result of an experiment found with the predictable result. The difference between the achieved result and the founded result is commonly divided by the predictable result and as a percent relative error was reported.

$$\% \textbf{ Error} = \frac{\textbf{obtained result} - \textbf{ expected result}}{\textbf{expected result}} \boldsymbol{x100}$$

Analytical methods may be divided into three groups based on the magnitude of their relative errors. When an experimental result is within 1% of the correct result, the analytical method is highly accurate. Methods resulting in relative errors between 1% and 5% are moderately accurate, but methods of low accuracy produce relative errors greater than 5%.

1.4.5. Precision

When a sample is analyzed several times, the individual results are rarely the same. Instead, the results are randomly scattered. Precision is a measure of this variability. The closer the agreement between individual analyses, the more precise the results.

Precision is a measure of the spread of data about a central value and may be expressed as the range, the standard deviation, or the variance. Precision is commonly divided into two categories: repeatability and reproducibility. Repeatability is the precision obtained when all measurements are made by the same analyst during a single period of laboratory work, using the same solutions and equipment. Reproducibility, on the other hand, is the precision obtained under any other set of conditions, including that between analysts, or between laboratory sessions for a single analyst. Since reproducibility includes additional sources of variability, the reproducibility of an analysis can be no better than its repeatability [57].

1.4.6. Quantification Limit (LOQ) and detection Limit (LOD)

The LOQ (Lowest amount of an analyte in a sample which can be determined quantitatively with an appropriate precision and accuracy) and LOD (lowest amount of an analyte in a sample which can be detected but not necessarily quantitated) and according to the International Conference of Harmonization (ICH) guidelines for validation of analytical procedures are determined. The following formulas were used: LOD or LOQ = k SDb/a, where k = 10 for LOQ and 3.3 for LOD, a is the slope and SDb is the standard deviation of the intercept [58].

1.4.7. Standard Deviation (SD)

The absolute standard deviation**,** SD, describes the spread of individual measurements about the mean, and is defined as a statistical measure of the "average" deviation of data from the data's mean value. SD is often expressed in a relative manner. Calculations are made therefore of the relative standard deviation (or RSD).

RSD = SD / X^{-}

Where X^{-} is the mean

RSD is multiplying by 100% to obtain percentage relative standard deviation

1.5. Role of Analytical Techniques in Pharmaceutical Analysis

Guided by pharmacology and clinical sciences, and driven by chemistry, pharmaceutical research in the past has played a crucial role in the progress of development of pharmaceuticals.The contribution of chemistry, pharmacology, microbiology and biochemistry has set a standard in the drug discovery where new drugs are no longer generated only by the imagination of chemists but these new drugs are the outcome of exchange of ideas between biologists and chemists [59].

Drug development processes were starts with the invention of a drug that has showed therapeutic value to conflicts, check, control, or cure diseases. The characterization and synthesis of such molecules which are also called active pharmaceutical ingredients (APIs) and their analysis to create therapeutic efficacy data and preliminary safety for further detailed investigations are precondition to identification of drug candidates [60].

In the field of pharmaceutical research, the analytical investigation of bulk drug materials, intermediates, drug products, drug formulations, impurities and degradation products, and biological samples containing the drugs and their metabolites is very important. From the commencement of official pharmaceutical analysis, analytical assay methods were included in the compendial monographs with the aim to characterize the quality of bulk drug materials by setting limits of their active ingredient content. In recent years, the assay methods in the monographs include spectrometry, chromatography, capillary electrophoresis and

titrimetry; as well the electroanalytical methods. The present state-of-theart is replicated through the data in Table 1.1 based on the edition of US (United States Pharmacopoeia, 2004) and European (The European Pharmacopoeia and Council of Europe, 2002) [61] pharmacopoeias.

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Table 1.1. Proportion of various analytical methods prescribed for the assay of bulk drug materials in Ph. Eur. 4 and USPXXVII [62].

Technique	Ph. Eur. 4 (%)	USP 27 (%)
UV–Vis spectrophotometry	9.5	8.5
Titration	69.5	40.5
GC	2	2.5
HPLC	15.5	44
Potentiometric	27	10
Non-aqueous	36.5	24
Aqueous mixtures	21	5.5
Redox (Iodometry, Nitritometry, etc.)	6.5	5.5
Microbiological assay (antibiotics)	3	2.5
Indicator	6.5	4.4
Indicator	9.5	14
Acid–base	57.5	29.5
Other (complexometry, argentometry, etc.)	5.5	5.5
Potentiometric	14.5	1
Other (atomic absorption spectroscopy ,IR, polarimetry NMR, gravimetry fluorimetry, polarography etc.)	0.5	2

1.5.1. Several Analytical Techniques that are Used in Analysis of Pharmaceuticals

- Titrimetric techniques
- Chromatographic techniques **:**

✓ Thin layer chromatography

✓ High performance thin layer chromatography

✓ High-performance liquid chromatography (HPLC)

✓ Gas chromatography

- Spectroscopic techniques

✓ Spectrophotometry

✓ Near infrared spectroscopy (NIRS)

✓ Nuclear magnetic resonance spectroscopy (NMR)

✓ Fluorimetry and phosphorimetry

- Electrochemical methods
- Kinetic method of analysis
- Electrophoretic methods
- Flow injection and sequential injection analysis
- Hyphenated techniques

1.5.2. The Role of Spectrophotometry and its Corresponding Analytical Method in the Analysis of Pharmaceuticals

One of the important groups of methods which find an important place in pharmacopoeias are spectrophotometric methods based on natural UV absorption and chemical reactions [63]. Spectrophotometry is the quantitative measurement of the transmission or reflection properties of a substance as a wavelength function.

The advantages of these methods are low time and labor consumption. The precision of these methods is also excellent. The use of UV–Vis spectrophotometry especially applied in

the analysis of pharmaceutical dosage form has increased rapidly over the last few years [64]. The colorimetric techniques are commonly based on the following aspects:

- Complex-formation reaction.
- A catalytic effect.
- Oxidation-reduction process.

It is important to mention that colorimetric methods are regularly used for the assay of bulk materials. For example, the blue tetrazolium assay is used for the determination of corticosteroid drug formulations [65]. The colorimetric method is also exploited for the determination of cardiac glycosides and is presented in European Pharmacopoeia. Several approaches using spectrophotometry for determination of active pharmaceutical ingredients in bulk drug and formulations have been reported and details of these methods are recorded in Table 1.2.

Table 1.2. Quantitative analysis of some drugs in pharmaceutical formulations by UV–Visible spectrophotometric procedures.

Reagent	Drug name	λmax	Reference
Quinalizarin	Ganciclovir	560	[70]
tetracyanoethylene (TCE)	Clotrimazole (CLZ)	396	[71]
7,7_,8,8_tetracyanoquinodimethane(TCNQ)	Clotrimazole (CLZ)	842	[71]
Quinalizarin	gabapentin (GAB)	571	[72]
alizarin red S (ARS)	gabapentin (GAB)	572	[72]
Quinalizarin	pregabalin (PRG))	528	[72]
alizarin red S (ARS)	pregabalin (PRG))	538	[72]
chloranilic acid	p-nitroaniline	530	[73]
2,5-dichloro-3,6-dihydroxy- benzoquinone	bifonazole	517	[74]
3,4-diaminotoluene	bifonazole	457	[74]

p-chloranilic acid	Amiodarone hyrdrochloride	535	[75]
1,2-naphthoquinone-4-sulphonate	modafinil	430	[76]
2,4-dinitrophenol	modafinil	475	[76]
Alizarin	desloratidine	528	[77]
Quinalizarin	desloratidine	560	[77]
Bromothymol blue	Rasagiline mesylate	414	[78]
Bromocresol green	Rasagiline mesylate	414	[78]
Chloranil	Dutasteride	525	[79]
Ninhydrin	Pregabalin	402.6	[80]
m-Cresol	Acetaminophen	640	[81]
Iodine	Aripiprazole	400	[82]

Derivative spectroscopy uses first or upper derivatives of absorbance with respect to wavelength for qualitative investigation and estimation. The concept of derivatizing spectral data was first offered in the 1950s, when it was shown to have many advantages. However, the technique received little consideration primarily due to the complexity of generating derivative spectra using early UV–Visible spectrophotometers. The introduction of microcomputers in the late 1970s made it generally convincing to use mathematical methods to generate derivative spectra quickly, easily and reproducibly. This significantly increased the use of the derivative technique. The derivative method has found its applications not only in UV spectrophotometry but also in infrared [66]. Fluorescence spectrometry,atomic absorption, [67], and fluorimetry [68].The use of derivative spectrometry is not limited to singular cases, but may be of benefit whenever quantitative study of normal spectra is difficult. Disadvantage is also related with derivative techniques; the differential damages the signal-to-noise ratio, so that some formula of smoothing is necessary in conjunction with differentiation [69].

1.5.3. The Role of Near Infrared Spectroscopy (NIRS) and its Corresponding Analytical Method in the Analysis of Pharmaceuticals

Near infrared spectroscopy (NIRS) is a rapid and non-destructive procedure that provides multi component analysis of almost any matrix. In recent years, NIR spectroscopy has gained a wide appreciation within the pharmaceutical industry for raw material testing, product quality control and process monitoring. The growing pharmaceutical interest in NIR spectroscopy is probably a direct consequence of its major advantages over other analytical techniques, namely, an easy sample preparation without any pretreatments, the probability of separating the sample measurement position by use of fiber optic probes, and the expectation of chemical and physical sample parameters from one single spectrum. The major pharmacopoeias have generally adopted NIR techniques. The European [83], and United States pharmacopoeias [84], ddress the suitability of NIR instrumentation for application in pharmaceutical testing. NIR spectroscopy in combination with multivariate data analysis opens many interesting perceptions in pharmaceutical analysis, both qualitatively and quantitatively. A number of publications describing quantitative NIR measurements of active ingredient in intact tablets have been reported [85]. In addition to the research articles many review articles have been published citing the application of the NIRS in pharmaceutical analysis [86].

1.6. Theory of Ultraviolet and Visible Absorption Spectroscopy

A diagram of the typical spectrometer components are shown in the (figure 1.4). The ultraviolet and visible (UV-Vis) absorption spectroscopy to measure the absorption of light is used when it is through a sample passed, in the visible and "near" ultraviolet area, that is in the 200-750 nm range. The ultraviolet and visible (UV-Vis) provides information about compounds with conjugated double bonds [87].

Ultraviolet light and visible lights have sufficient energy to the promotion of an electron from one orbital to another of higher energy (electronic transition), dependent on the energy required for the electronic transition, a molecule either ultraviolet or visible region will absorb light. A UV spectrum is achieved if it absorbs ultraviolet light, a visible spectrum is achieved if it absorbs visible light. Ultraviolet light is electromagnetic radiation with wavelengths ranging from 180 to 400 nm (nanometers), the wavelengths of visible light is ranging from 400 to 780 nm, (One nanometer is or10 Å.) .Wavelength (λ) is inversely related to the energy, The shorter the wavelength, the greater is the energy. Visible light has smaller energy than Ultraviolet light.

$E = hc/\lambda$

Where: c velocity of light and λ wave length

h : Plank's constant

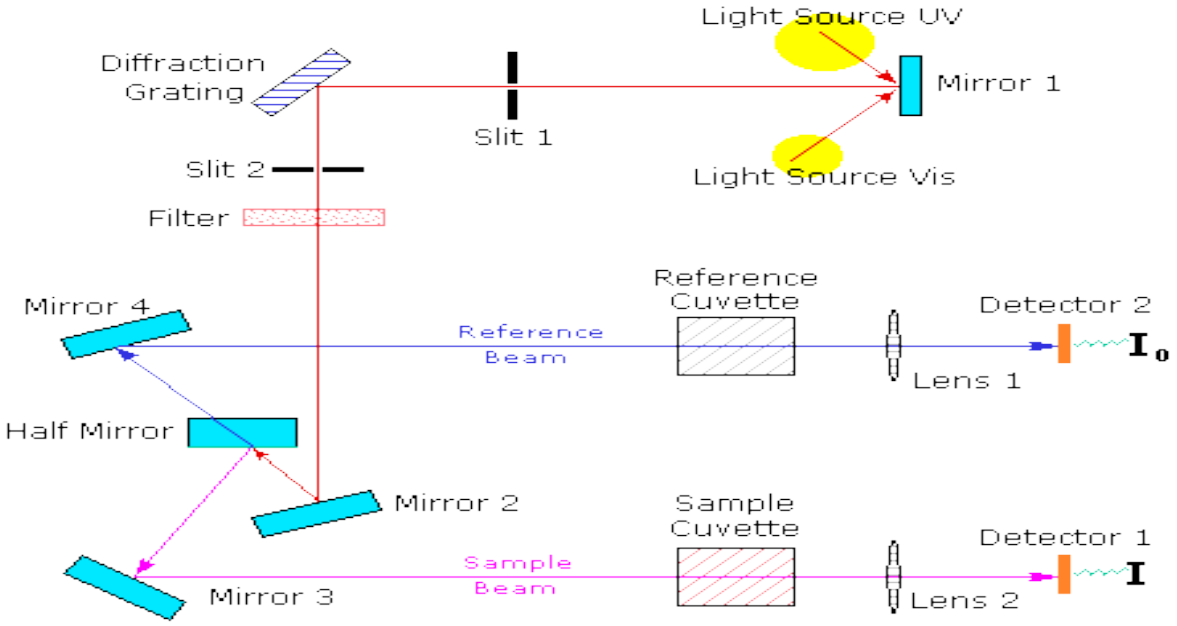

Figure 1.4. A diagram of the components of a typical UV/Visible spectrophotometry.

Many types of transitions between quantized energy levels account for molecular UV/Vis spectra. S-electron is held tightly, and a good deal of energy is required to excite it: energy corresponding to ultraviolet light of short wavelength, in a region "far" ultraviolet - outside the range of the usual spectrometer .It is chiefly excitations of the comparatively loosely held *n* and π-electrons that appear in the (near) ultraviolet spectrum, and of these, only jumps to the lower more stable excited states (Figure 1.5).

The electronic transitions of most significant are:

(a) n → π *, in which the electron of an unshared pair goes to an unstable (antibonding) porbital.

(b) π → π*, in which an electron goes from a stable (bonding) porbital to an unstable (antibonding) porbital.

Because they include functional groups that are representative of the analyte, and wavelengths that are easily available [87].

The approximate wavelength ranges for these absorptions, as well as a partial list of bonds, functional groups, or molecules that give rise to these transitions is shown in (Table 1.3). The specific bonds or functional groups of organic compounds (ketones, amines, nitrogen derivatives, etc.), responsible for the absorption of a particular wavelength of light in UV/Vis are called chromophores [88].

Table 1.3. Electronic transitions involving n, s, and p molecular orbitals.

Transition	Wavelength range (nm)	Examples
n → s *	< 200	C–C, C- H
n → s *	160- 260	H_2O, CH_3OH, CH_3Cl
π → π*	200- 500	C=C, C=O, C=N, C= C
n → π*	250- 600	C=O, C=N, N=N, N=O

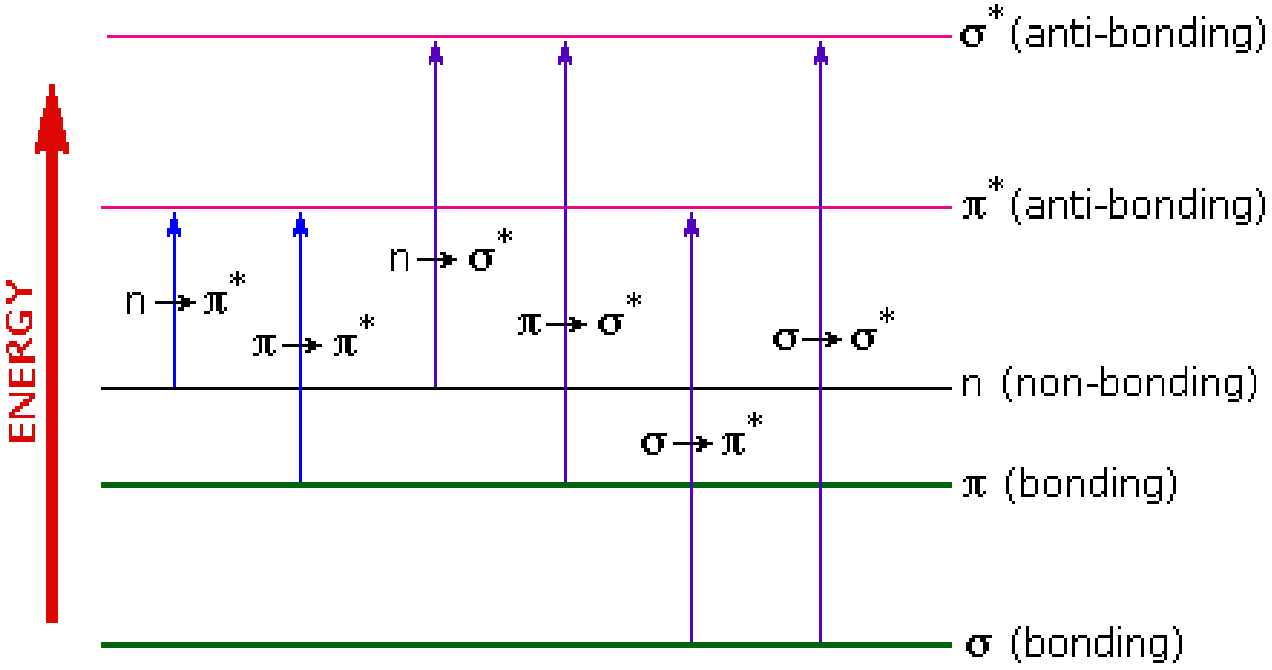

Figure 1.5. Electronic energy level and transition state.

The basis of spectrophotometric methods is the simple relationship between the color of a substance and its electronic structure. A molecule or an ion exhibits absorption in the visible or ultra-violet region when the radiation causes an electronic transition in molecules containing one or more chromophoric groups. The color of a molecule may be intensified by substituents called auxochromic groups, which displace the absorption maxima towards longer wavelength (bathochromic shift). The color determining factors in many molecules is the introduction of conjugated double bonds by means of electron donor or electron acceptor groups [89].

The quantitative applicability of the absorption method is based on the fact that the number of photons absorbed is directly proportional to the number or concentration of atoms, ions or molecules. Absorption spectroscopy is one of the most useful tools available to the chemist for quantitative analysis. Important characteristics of spectrophotometric methods include:

- Wide applicability: Numerous inorganic and organic species absorb in the ultraviolet and visible ranges and are thus susceptible to quantitative determination.
- High sensitivity: Having calibration graphs that are linear over a wider range.Very extensive range of concentration of substances (10-2 –10-8 M) may be covered.
- Moderate to high selectivity: It may be possible to locate a wavelength region in which the only absorbing component in a sample is the substance being determined.
- Ease and convenience: Spectrophotometric measurements are easily and rapidly performed with modern instruments [90].

1.7. Beer's Law in Chemical Analysis

The Beer – Lambert law to determine the concentration of analyte is used by measuring the absorbance at several wavelengths. Beer – Lambert law is the correlation between the absorbance of sample and the concentration of the absorbing species. It can be written as:

A = e bc

'*A*' is absorbance,

'*c*' is the concentration of the sample (compound) and expressed as mole/L

'*b*' is pathlength of cell and expressed in units cm.

'*e* ' is the molar absorbtivity (or extinction coefficient in the older literature) and has the units $L\ mol^{-1}.\ cm^{-1}$.

Beer-Lambert law offers a valuable and simple method for quantitative analysis. In practice, a calibration curve is constructed by plotting absorbance vs. concentration and the concentration of unknown with 'X' absorbance is determined by finding the concentration corresponding to the measured absorbance on the calibration curve [91].

1.8. Summary of Literature Review

Several techniques for the modafinil determination have been described. Amongst them, the methods that are used in biological fluids for the quantification of modafinil are high performance liquid chromatography [92,93]. Gas chromatography–mass spectrometry[94] and liquid chromatography-mass spectrometry[95]. In bulk and tables formulation modafinil was determined through methods such as high performance liquid chromatography[96,97], and high-performance thin layer chromatography[98]. The above reported chromatographic methods utilize sophisticated and high cost instrumentation that are generally not available in most of the quality control laboratories of underdeveloped and developing countries. As a result, the applications of these techniques for the quantification of modafinil in biological samples, pure and tables formulations are limited. Spectrophotometry may serve as a convenient alternative technique to the abovementioned advanced chromatography techniques owing to its simple operation cost efficacy, fair accuracy, sensitivity, precision and wide-ranging applicability.

A few spectrophotometry approaches have been described for the quantification determination of modafinil in bulk and pharmaceutical formulations among them.

Venkatesh et al [99] was reported UV spectrophotometric technique for the determination of modafinil in pharmaceutical dosage. It involves absorbance measurement at 236 nm in glacial acetic acid medium. However, the UV spectrophotometric method suffers from main drawbacks. These drawbacks comprise reduced selectivity due to measurement in UV region, narrow range of linearity, lack of precision and accuracy .

C.B. Sekaran et al. [100] have developed Two spectrophotometric methods for the estimation of modafinil in the method one used 1,2-naphthoquinone-4-sulphonic acid(NQS) as analytical reagent and in method two used 2,4-dinitrophenolin(DNP) as analytical reagent.The method one (NQS method) are based on the nucleophilic substitution reaction between modafinil drug and chemical reagent in alkaline medium to produce an orange yellow colored product that showing maximum absorption at 430 nm and linear range over 10-100 µg/mL. The method presented a limit quantification of 1.620 µg/ml and a limit of detection of 0.486 µg/ml. In method two(DNP as lewis acid) react with amino group(as lewis

base) of modafinil at room conditions to form yellow colored stable ion pair complex as a consequence of a proton transfer from hydroxyl group of lewis base , 2,4-dinitrophenol reagent to the primary amino group of lewis base (modafinil). The colored ion pair complex have maximum absorption at 475 nm. Beer's law is obeyed in the concentration range 8-60 μg/mL **.**

N.G. Rashmi et al. (101) have described two spectrophotometric methods that are free from extraction steps for the quantitative assessment of modafinil in pharmaceutical formulations and pure form in (method I) used 2,4- dinitrophenyl hydrazine(2,4-DNP) as analytical reagent .This method is based on the oxidation of reagent (2,4-DNP) at first by KIO3 to form diazonium cation and then this oxidized product is coupled with modafinil drug by electrophic substitution to form deep coloured chromogen that have maximum absorption at 470 nm and analytical calibration curve in the concentration range of (2.0-7.0 $\mu g.ml^{-1}$).In method(II) used p-dimethyl amino cinnamaldehyde (PDAC) as reagent .The reaction is based on the condensation between (PDAC) reagent and modafinil in acidic medium to procedure reddish brown coloured product instantaneously that showed maximum absorption at 443 nm and linearity of the concentration range is (6.0-14.0 $\mu g.ml^{-1}$) .The recovery of two methods found to be 99.9 and 99.6 respectively .

Seshamamba et al. [102] have reported two methods for quantification of modafinil in dosage formulation and bulk drugs by visible spectrophotometric. The methods (I and II) are based on the bromination of modafinil by a bromate/bromide mixture of known concentration in acid medium .The ability of bromine residual to bleach the methyl orange and methylene blue in method I and II used to determine the amount of bromine and there absorbance measured at 525 nm and 664 nm in both methods respectively.In both methods the reacted amount of bromine with dye relates to the content of modafinil drug.

2. MATERIALS AND METHODS

2.1. Apparatus

All the absorption spectral measurements were made using T80plus UV–Vis double beam spectrophotometer (PG instrument Ltd) with 1cm matched quartz cells. The infrared spectra for the solid charge transfer products were recorded within the range of 4000–400 cm^{-1} on a perkin-elmer spectrum one FT-IR spectrometer.

2.2. Reagents and Solutions

All employed solvents (methanol, ethanol, dimethyl sulfoxide, acetonitrile, and acetone) used in this work were of analytical grade. Modafinil reference standard and bulk powder was kindly provided by (GENERICA ILAC SAN VE TIC A.S., TURKEY). Its purity was found to be 99.78% according to the manufacturer's method and used as received, pharmaceutical dosage form used included modiodal tablets (product of Tava Pharma.B,Turkey), labeled to contain 100mg of modafinil per tablet was purchased from commercial source .

2.2.1. Standard Solutions

A standard stock solution of the studied drug containing 1000 $\mu g.mL^{-1}$ was prepared by accurately weighing approximately 0.1 g of pure drug and dissolving in 50 mL of methanol. The solution was then transferred to 100 mL standard flasks and diluted to the mark with the same solvent to obtain the working concentration.

2.2.2. Reagent Solution

Quinalizarin reagent was Aldrich product and was used as received without further purification provided by firat university. 1.0×10^{-3} M of reagent solution was freshly prepared by dissolving the appropriate weight of the reagent in approximately 25 mL of methanol, then the solution was completed to the mark with same solvent in 100 mL volumetric flask. This solution was stable for at least for one weak.

2.3. Construction of Calibration Curves

Aliquots equivalent to (20-180µg.mL^{-1}) of standard working solution were accurately transferred into a series of 10 mL calibrated volumetric flasks, then to each flasks 1mL of (1.0 x 10^{-3}M) chromogenic reagent (quinalizarin) solution was added. Afterward, the obtained mixture was shaken in order to promote the reaction and the volume was made up to the mark with methanol ,the flasks ware kept at room temperature for 20 minutes .Then the absorbance of the violet colored product at 572 nm was measured against a reagent blank containing only the quinalizarin reagent. Perform a linear regression analysis using absorbance data against final concentration of the modafinil in µg.mL^{-1} . In unknown sample the amount of modafinil was calculated by either use the corresponding regression equation or corresponding calibration curve (figure 2.1)

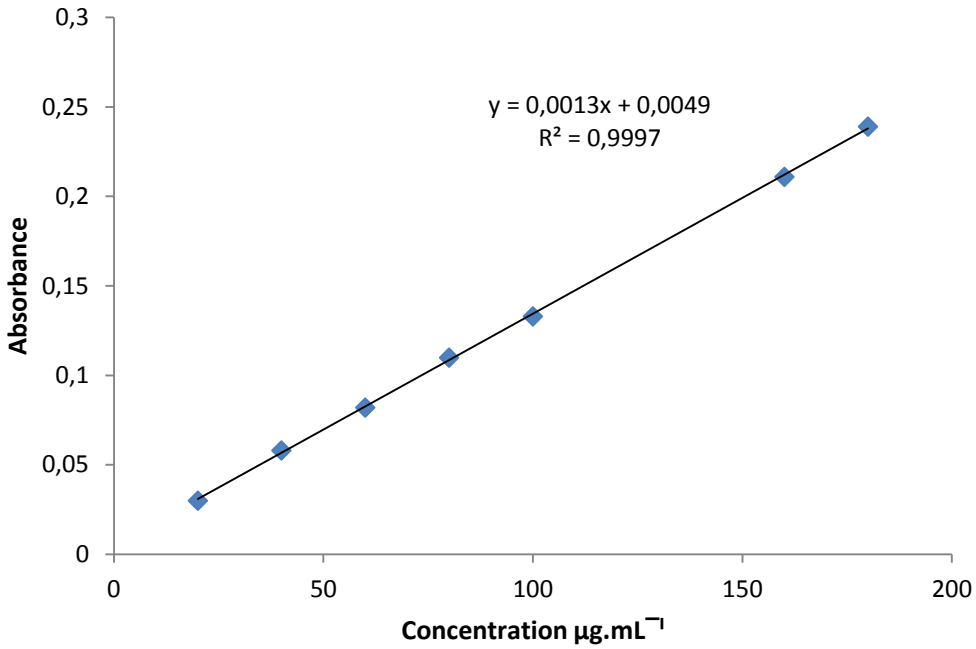

Figure 2.1. Calibration curve of modafinil.

2.4. Determination of Stoichiometry of the Complex

The stoichiometric ratios of the charge transfer Complex formed between modafnil and quinalizarin reagent is determined by applying the continuous variation method attributable to Job's method [103] and modified by Vosburgh and Coober [104]. At the optimum wavelength of maximum absorbance. Job's method of continuous variation was employed. (1.0×10^{-3} M) standard solution of modafinil and (1.0×10^{-3} M) solution of quinalizarin reagent were used . A series of solutions were prepared in a 10 mL calibrated flask by mixing different complimentary proportions of drug and reagent in which the total volume of reagent and drug were kept at 2.0 mL. Then the solutions were made up to the mark with the same solvent and analyzed according to mentioned procedures. The absorbance of formed solutions was measured at their optimum wavelength of absorption against a reagent blank prepared similarly.

2.5. Application to Pharmaceutical Formulations (Tablets)

The tablet formulation of modafinil, (modiodal) labeled to contain 100mg per tablet were purchased from a local pharmacist. Mass of each tablet independently was measured (approximately 250 mg each tablet) .The contented of ten tablets were finely powdered in an agate mortar. A portion of the powdered sample equivalent to 50 mg modafinil was accurately weighed and transferred to a 50 mL beaker, 30 mL of methanol was added and the mixture was shaken for 20 minutes. The mixture was filtered into 100 mL volumetric flask through a PTFE membrane filter with 0.2μm pore diameter.The solution was made up to the mark with methanol to obtain the stock solution ($1000 \mu g.mL^{-1}$). This solution was further diluted with the same solvent as appropriate to obtain the working concentration ranges. Aliquots covering the working concentration ranges were transferred into a series of 10 mL volumetric flasks and the proposed method was applied. The nominal contents of the tablets were determined either from using the corresponding regression equation or the calibration graph. The developed procedure for the analysis of modafinil in commercial formulation sample (modiodal tablets) was applied .

2.6. Synthesis of Solid Charge Transfer Complex

Infrared spectra of the modafinil drug, quinalizarin reagent and reaction product of their mixture (complex) were recorded as potassium bromide pellets. For this purpose, approximately 10 mg of each compound were mixed with 100 mg of KBr and pressurized to obtain a pellet. Then, the pellet was transferred to the spectrometer and the spectra were recorded in the range of 4000-400 cm^{-1} with a nominal resolution of 4 cm^{-1}.

For the synthesis of the solid charge transfer complex (reaction product). Equimolar saturated solutions of the modafinil and quinalizarin in two separate flasks were prepared in methanol solvent and mixed simultaneously. After a clear solution was obtained, at low temperature and pressure the solvent was evaporated and the residual solid product was used for preparation of KBr pellet and posterior FT-IR spectrum recording.

3. RESULTS AND DISCUSSION

3.1. Absorption Spectra

Quinalizarin reagent was employed for the determination of modafinil drug. The procedure depends on the formation of charge transfer complex upon the reaction of the reagent with modafinil in alcoholic medium. The reaction proceeds through the formation of a colored charge transferred product, which was measured spectrophotometrically.

The development and study of the method for the modafinil determination in pharmaceutical formulations and bulk powder, exploring its charge transfer reaction with quinalizarin was performed through two steps: (i) optimization of the experimental conditions in order to achieve both maximum sensitivity and selectivity. This step comprised the evaluation of the effect of the solvent nature, investigation of the influence of the reagent concentration and evaluation of the time required to complete the reaction and; (ii) study and characterization of the reaction, which were carried out by the evaluation of the stoichiometry of the reaction (Job's continuous variation method) and the verification of the proposed reaction mechanism.

At optimum conditions, the radical anions (absorbing species) were formed in the medium after mixing of the reagent and showed maximum absorption at 572 nm, in methanol medium (Figure 3.1). Thus, this wavelength was chosen for all further measurements in order to obtain highest sensitivity for the proposed method. It is important to point out that in the methanol quinalizarin showed maximum absorption at 491 nm and drug at 250nm (Figure 3.2, 3.3). The high difference between maximum of the charge transfer complex product and reagent absorption band 81 nm, allowed the measurement of the product with only a small contribution of the reagent that was added in excess in the medium.

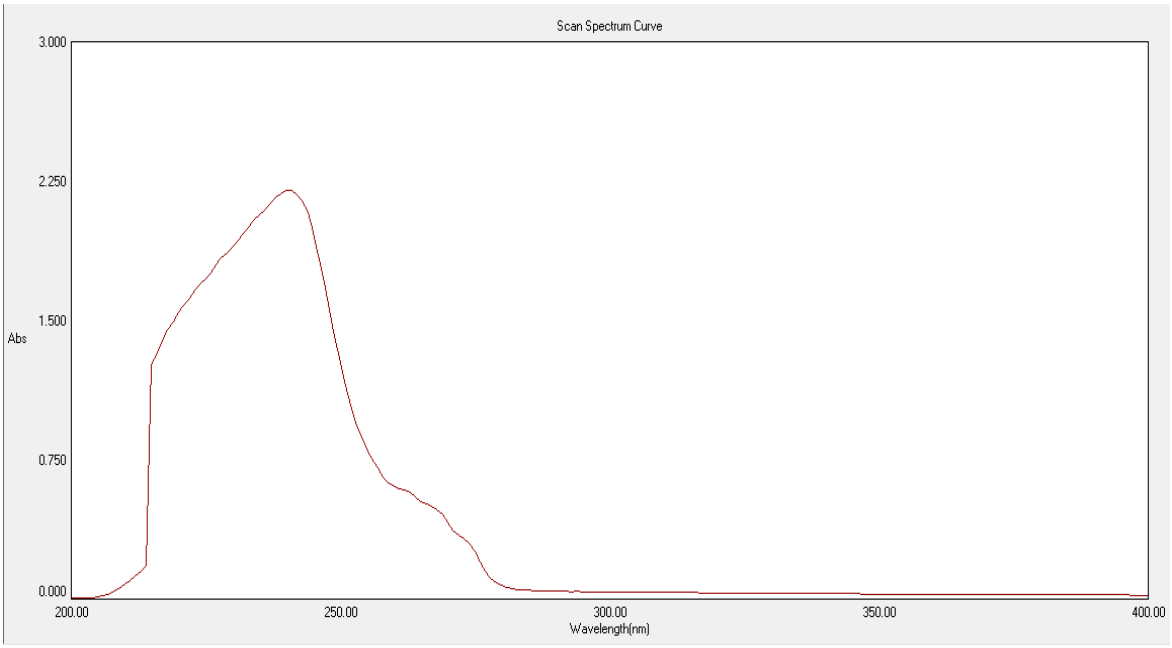

Figure 3.1. Absorption spectra of modafinil (1000µg.mL^{-1}) against methanol.

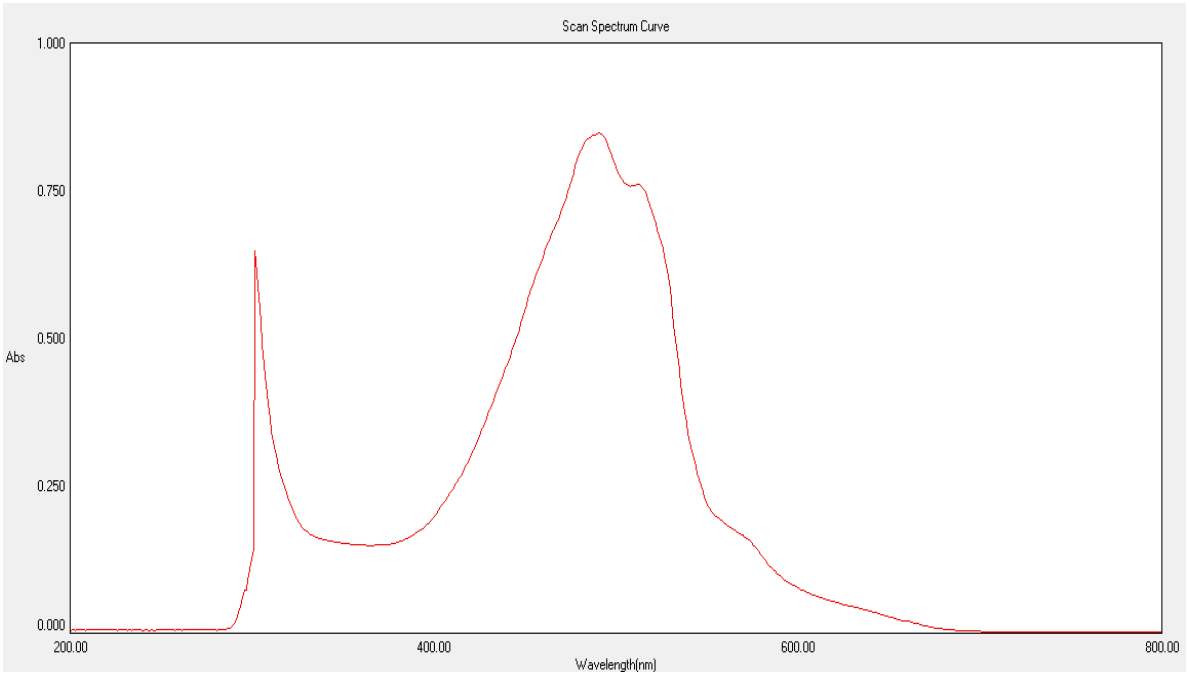

Figure 3.2. Absorption spectrum of quinalizarin (1.0 x 10^{-3} M) against methanol.

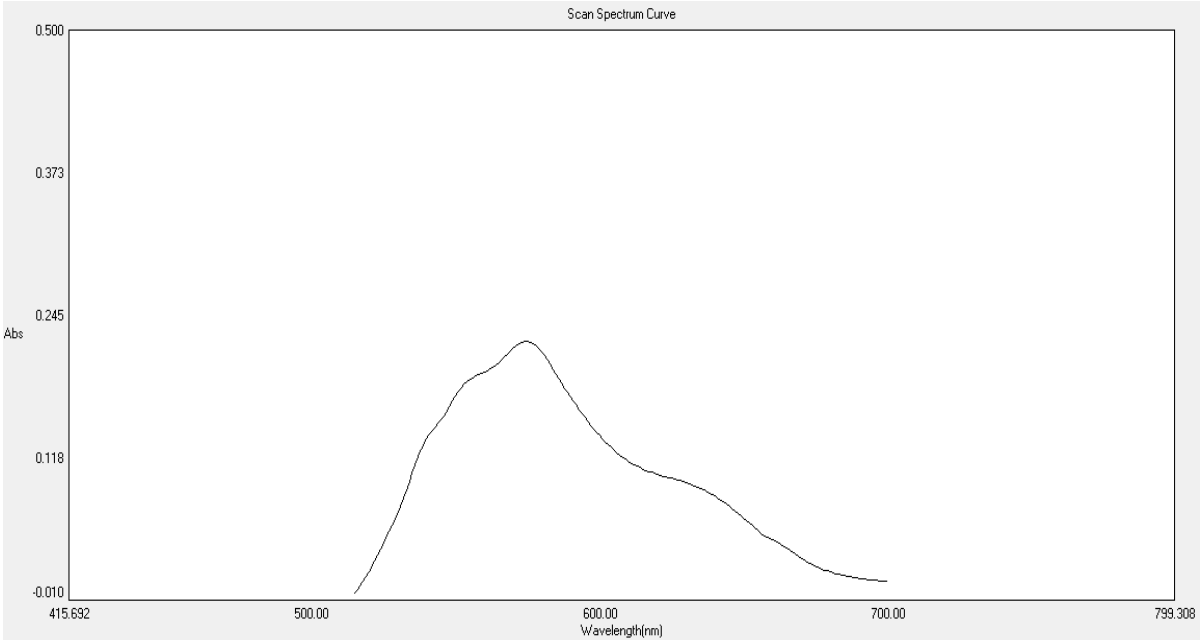

Figure 3.3. Absorption spectra of charge transfer complex of modafinil (180µg.mL^{-1}) with 1mL quinalizarin (1.0 x 10^{-3} M).

3.2. Optimization of Experimental Conditions

Investigation was carried out to establish the most favorable conditions to give a highly intense color and to achieve maximum color development in the quantitative determination of the examined drug. The influence of each of the following variables on the reaction was tested.

3.2.1. Effect of Solvent Nature

The first parameter evaluated in the optimization of the experimental condition was the nature of the solvent employed. In the some charge transfer reactions the solvent plays an important role .Since it must be able to facilitate the total charge transfer and then allow the complex dissociation and stabilization of the radical anion formed, which is the absorbing species. According to the literature, solvents with high dielectric constant are more effective to execute this task [105]. Taking this fact into account, water would be an excellent solvent for

the procedure. However, the poor solubility of the reagent in water did not allow its use in the present case. So, the reaction was tested in methanol, dimethyl sulfoxide (DMSO), ethanol, acetonitrile and acetone media. Although the highest dielectric constant of DMSO and acetonitrile ,best sensitivity was achieved with methanol, probably because of the capacity of this solvent to form hydrogen bonds with the radical anion. Then, methanol was selected for further experiments (Figure 3.4).

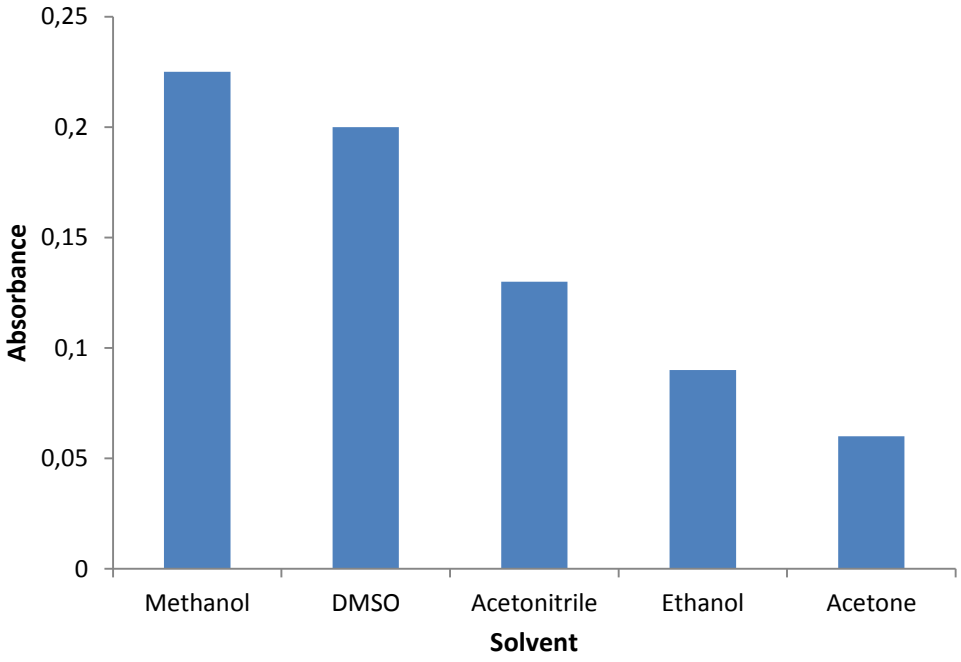

Figure 3.4. Effect of different solvents on the charge transfer complex of drug - quinalizarin complex obtained against reagent solution also prepared in each solvent. Modafinil concentration = (180µg. mL^{-1}) ,1mL reagent concentration (1.0 x 10^{-3}M).

3.2.2. Effect of the Reagent Concentration

In spectrophotometric analytical methods where maximum sensitivity is desired, the reagent concentration in solution is an important parameter to be studied, since the maximum conversion of the analyte into absorbing species depends on the amount of the reagent available in the solution for reaction and the equilibrium involved In order to achieve this objective, an experiment was performed when various concentrations of reagent solution (1.0 x 10^{-3} M) in the range of 0.2– 1.8 mL were added to a fixed drug concentration (180 µg.mL^{-1}) (figure 3.5). The results showed that 1.0 mL of (1.0 x 10^{-3} M) reagent solution was enough to develop the color to its full intensity. As it can be seen, remarkable increase of the absorbance was verified up to 1.0 mL, after this point, it only suffered a slight increase or constant absorbance.

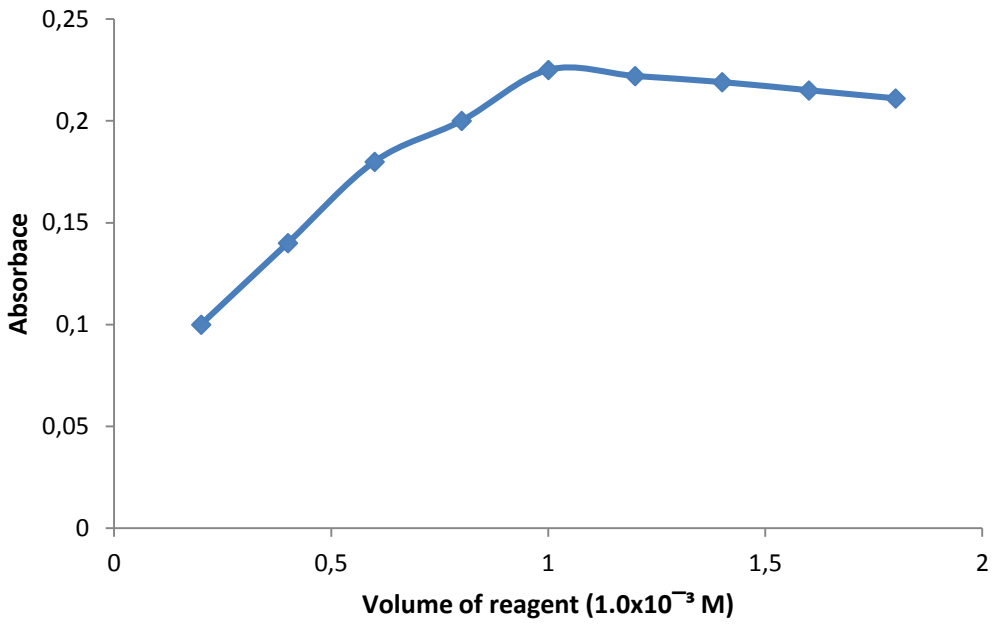

Figure 3.5. Effect of (1.0 x 10^{-3} M) quinalizarin concentration on the absorbance of charge transfer complex formed between drug (180µg. mL^{-1}) and reagent at the optimum wavelength .

3.2.3. Effects of Time and Temperature

The optimum reaction time was determined by continuous monitoring of the absorbance at optimum wavelength of a solution containing 180 µg.mL^{-1} modafinil and 1.0 mL of (1.0 x 10^{-3} M) reagent, at laboratory ambient temperature (25 ±2 C). On raising the temperature, the absorbance of the charge transfer complex was slight decreased with a hypochromic shift, until decayed at 55 ºC. Maximum absorbance was observed after 20 min from the starting point of the experimentation up to 4 hours. After this time, absorbance suffered a small decrease In view of these results, all spectral measurements were carried out after 20 min of mixing of the reagents and 25± 2 ºC in order to make the method speedy (figure 3.6.,3.7).

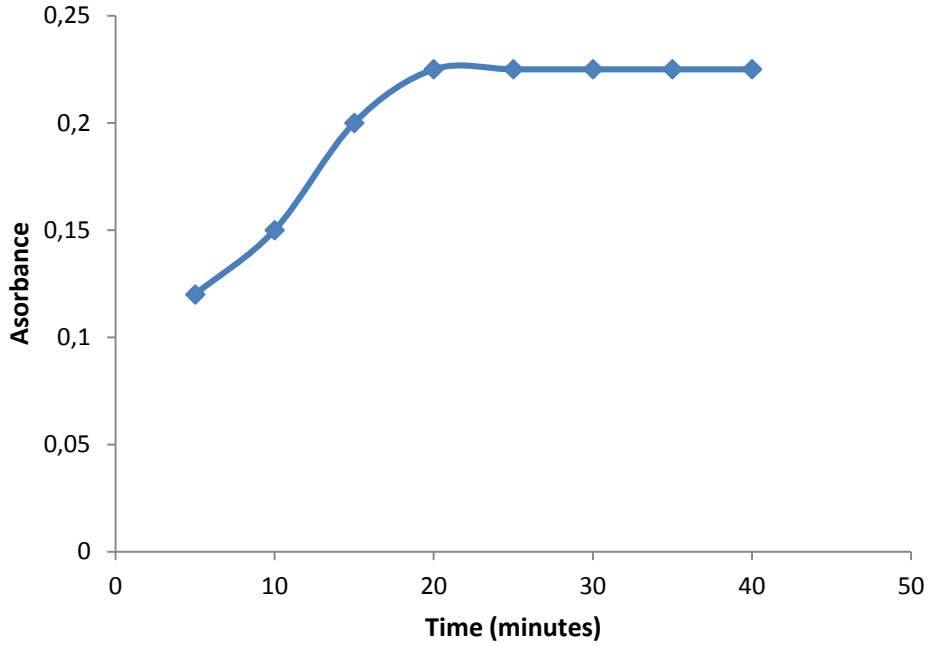

Figure 3.6. Effect of time on the absorbance of reaction modafinil (180µg. mL^{-1}) with quinalizarin.

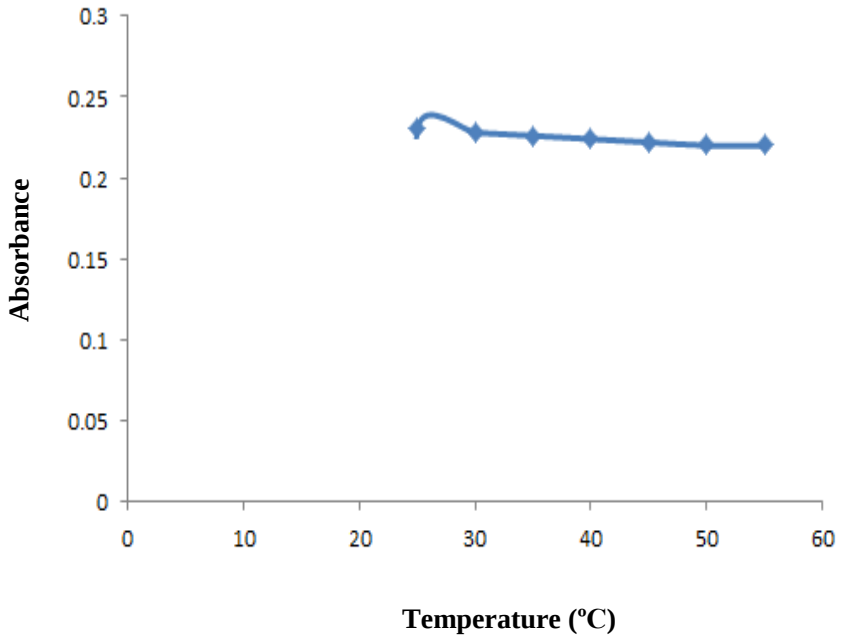

Figure 3.7. Effect of temperature on the absorbance of reaction modafinil (180 µg. mL^{-1}) with quinalizarin.

Table 3.1. Optimum conditions for the reaction.

Condition	Value
Temperature (ºC)	25 ± 2
Time of reaction (minutes)	20
Volume of reagent (mL)	1.0

3.3. The Stoichiometry of the Charge Transfer Complex

Job's method of the continuous variation [106] was employed to determine the stoichiometry of the charge transfer reaction in methanol medium. Keeping the sum of the molar concentrations of the examined drug (modafinil) and reagent fixed, in the mixture the ratio of the concentrations of the drug and reagent was varied and the absorbances of the mixtures were recorded at optimum wavelength against a convenient blank solution prepared for each point of the experiment. As shown in (Figure 3.9), the modafinil molar ratio which give best absorbance was 0.5, representing that it react with π-acceptor reagents in a proportion of (1:1) (Drug : Reagent) .In view of this result a reaction mechanism was suggested considering that free electron from the nitrogen atom existing in molecule of modafinil transferred to the charge-deficient position of the quinalizarin molecule.

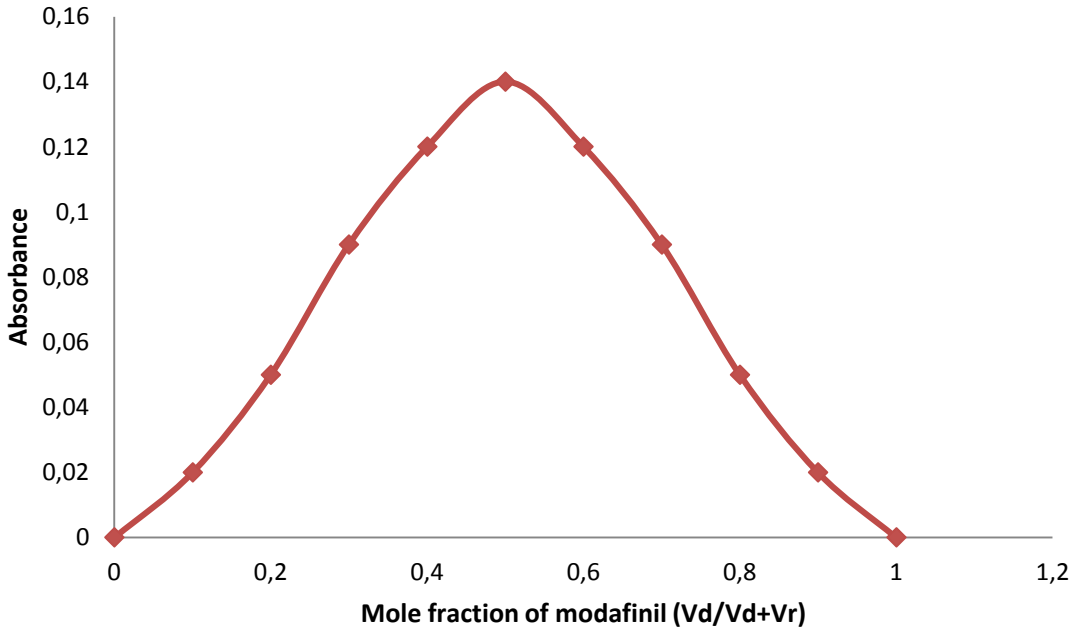

Figure 3.8. Job's method of continuous variation between modafinil and quinalizarin.

3.4. Mechanism of the Reaction

Solution of quinalizarin reagent in methanol exhibit an absorption band with a well-defined maximum at 491 nm, while the drug solution in methanol showed no absorption in the 400–700 nm range. The addition of drug to the reagent solution in methanol caused an immediate change in the absorption spectrum with the appearance of a new characteristic band with maximum absorption at optimum wavelength recorded in table 3.2.

According to (Ayad et al., 1984) molecular complexes of charge transfer are formed in non-polar solvents whereas radical anion species are predominant in polar solvents. Also, it is believed that the addition of basic compounds that contain a lone pair of electrons, such as modafinil, results in the formation of charge transfer complexes of n–π type. This type of complexes can be measured as an intermediary molecular-association compound that forms a corresponding radical anion in polar solvents. In this case, radical anions result from the total transfer of charge (Figure 3.9).

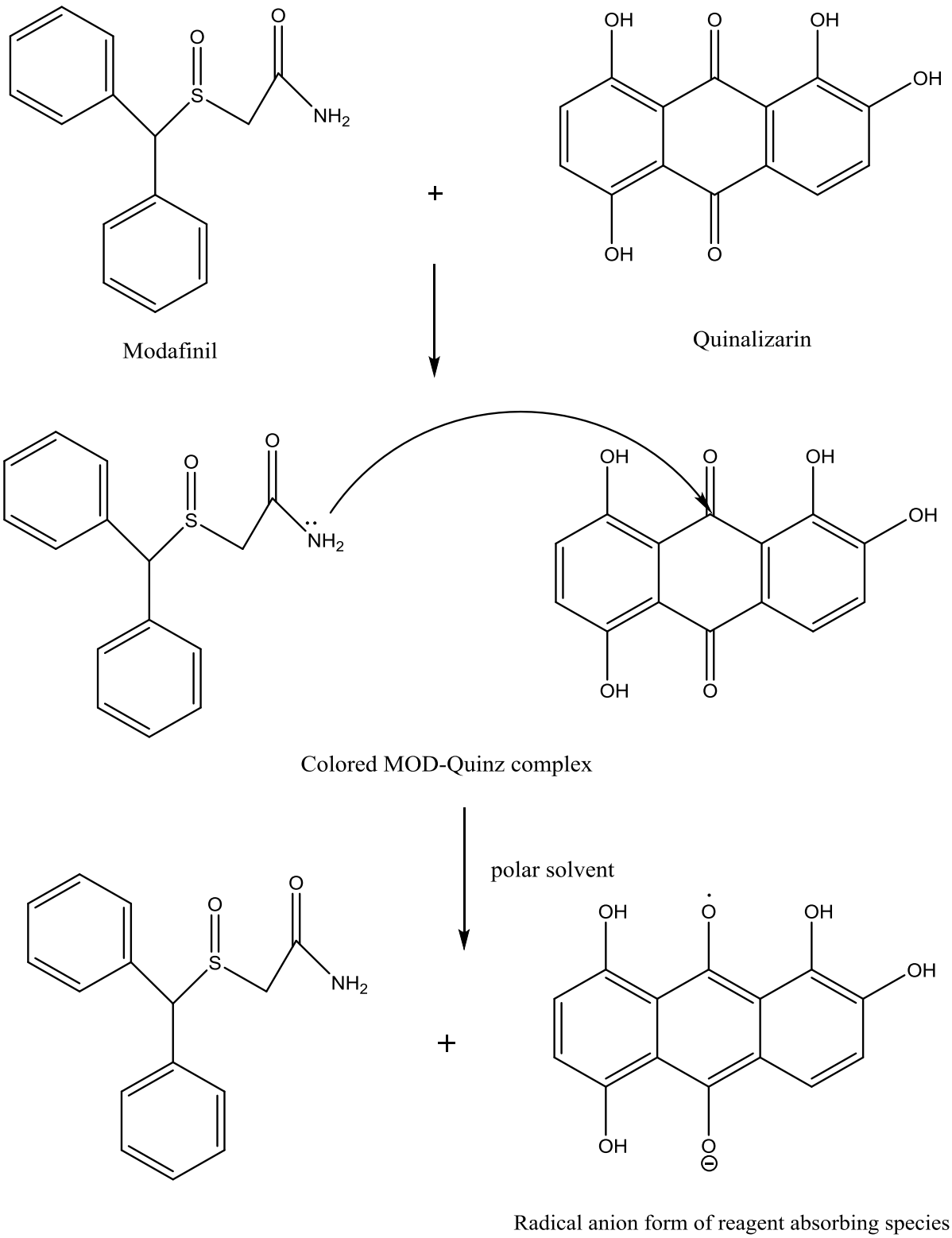

Figure 3.9.reaction of modafinil with quinalizarin.

3.5. Validation Method

The validation of the proposed method was tested according to the ICH guidelines for validation of analytical procedures [107], which included the evaluation of linearity, sensitivity, accuracy, precision, Ruggedness and Robustness, and limits of detection and quantification.

3.5.1. Linearity

By using the above procedure, linear regression equation was obtained. The regression plots showed that there was a linear dependence of the analytical response in the method to the concentration of the drug over the range cited in Table 3.2. Linear regression analysis of the data gave the following equation. A = 0.0013 X + 0.0049, r = 0.9997. Where A is the absorbance, X is the concentration of the drug ($\mu g.mL^{-1}$), and r is the correlation coefficient. Other statistical parameters, namely intercept (b), slope (a), molar absorptivity values are calculated as well and given in table 2.3.

Table 3.2. Analytical parameters for the determination of the modafinil by the proposed method.

parameters	values
Wavelength (λ max)	572
Range of concentration ($\mu g.mL^{-1}$)	20-180
Correlation coefficient(r)	0.9998
Regression equation [a]	Y=0.0013X+0.0049
Slope	0.0013
Intercept	0.0049
Detection limits ($\mu g.mL^{-1}$)	1.1169
Quantification limit ($\mu g.mL^{-1}$)	3.384615
molar absorptivity ($L.mol^{-1}.cm^{-1}$)	$0.44x10^2$

[a] Y =aX + b as X is concentration of modafinil in $\mu g.mL^{-1}$,Y is the absorbance , a is the slope and b is the intercept .

3.5.2. Limits of Detection and Quantification

The limit of quantification (LOQ) was determined by establishing the lowest concentration that can be measured according to ICH [107]. The limit of detection (LOD) was determined by establishing the minimum level at which the analyte can be reliably detected. The results are summarized in (table.3.2.). LOQ and LOD were calculated according to the following equations:

$LOQ = 3.3 (SD / a)$

$LOD = 10 (SD / a)$

Where **a** is the slope of the calibration curve, **SD**: standard deviation of the y-intercept of regression line**.**

3.5.3. Accuracy and Precision

The accuracy and precision of the proposed method were evaluated by analyzing the pure drug at three different concentration levels within the working range, each concentration being replicated five times, and the results are presented in Table 3.3. The percentage relative standard deviation (% RSD) value was less than 2% for the method, indicating good precision of the developed method. The accuracy of the proposed method was evaluated as the percentage relative error (RE%), and the value was less than 1%, demonstrating good accuracy for the proposed method.

Table 3.3. Evaluation of the accuracy and precision of the proposed method .

Taken Amount ($\mu g.mL^{-1}$)	found Amount[a] ($\mu g.mL^{-1}$)	RE[b] (%)	SD[b] ($\mu g.mL^{-1}$)	RSD[b] (%)	Recovery (%)
40	41.7	0.72	0.63	1.13	104.4
100	99.46	0.20	0.87	0.73	99.46
180	181	0.34	1.37	0.82	100.5

[a] Mean value of five determinations.
[b] RE – relative error; SD – standard deviation; RSD – relative standard deviation.

3.5.4. Ruggedness and Robustness

The robustness of the proposed method was examined by evaluating the influence of small variations in the procedure variables, such as time of the reaction (20 ± 5 minutes), added reagent volume (1.0 ± 0.1 mL), and using a different instrument, by two different analysts under the same optimized conditions. The obtained reproducible results (Table 3.4) showed that none of these variables and changes significantly affected the assay of modafinil.

Table 3.4. Results from robustness tested .

Changed parameter		Recovery (%)	RSD (%)
Added reagent volume	1.0 + 0.1	99.8	0.673
	1.0 – 0.1	99.6	0.564
Time of reaction	20 + 5	100.05	0.822
	20 – 5	99.8	0.763

3.5.5. Specificity

The effect of common excipients added as additives in the formulation is experimentally studied. In this approach, a known concentration of modafinil was prepared and it was spiked with three different concentrations of additives such as Lactose Monohydrate, Magnesium stearate, Croscarmellose sodium, and Povidone (K-30), and the absorbance of the resulting solutions were recorded. The mean percentage recovery values given in Table 3.4 indicate no potential interference from the excipients, which confirm the specificity of the proposed method.

Table 3.5. Recovery of modafinil in the presence of excipients.

Excipient	Mean recovery (%)
Lactose Monohydrate	99.8
Magnesium stearate	99.92
Povidone (K-30)	100.23
Croscarmellose sodium	100.43

3.5.6. Application to Analysis of Formulation

The suggested method can be used for modafinil determination in pharmaceutical dosage forms and the results obtained are given in Table 3.6. The statistical value of the t-test achieved at 95% confidence level, indicate that the suggested charge transfer complexation method are reliable and accurate. The high percentage recovery value shows that this method has the advantage of being potentially free from excipients.

Table 3.6. Statistical results of the assay of formulation by proposed method.

Trademark name	Branded amount (mg)	Amount found[a] ± SD
Modiodal	100 mg	99.46±1.2
		t-test[b] = 2.34
		% Rec[c]=99.46

[a] Mean value of five determinations.
[b] Theoretical t value at 95% confidence level is 2.7.
[c] Recovery.

3.6.Characterization of reaction product

3.6.1. Infrared Spectra

Infrared spectra of the drug, reagent and as well as resulting charge transfer complex are shown in (Figure 3.10,3.11 and 3.12), and their band assignments are reported in Table 3.7. The donation process from donor modafinil to the π-acceptor can occur from the lone pair of electron on the nitrogen atom of amino group shown in figure 3.9.A comparison of the relevant IR spectral bands of modafinil with the acceptor quinalizarin clearly indicated shifts in the frequencies as well as reductions in the band strengths, which proved the charge transfer complex formation. These shifts in the values were due to changes in the electronic structures and molecular symmetries of reactants upon complex formation [23]. The spectrum of the quinalizarin alone (Figure 3.11) presented some specific absorption bands at 1457 and 1271 cm^{-1}, which can be assigned to the stretching of the two C=O bonds present in its structure. After reacting quinalizarin with modafinil, these bands almost disappeared (Figure 3.12), showing that the C=O group was not present in the molecule anymore. This change in the spectrum evidenced the formation of a radical anion of quinalizarin from total charge transfer of the modafinil.

Table 3.7. Characteristic infrared frequencies (cm^{-1}) for modafinil ,quinalizarin and charge transfer complex.

Compound	Frequency	Assignments
Modafinil	1686 vs	ν (C=O)
	1033 ms	ν (S=O)
	1632w	δ (NH_2)
Quinalizarin	1271 s	δ (C–O)
	1235 m	ν (C–O)
	1457 vs	ν (–OH)
Complex	1622 m	ν (NNH2+) def
	1686 vs	ν (C=O) def

[a] m: medium; s: strong; v: very; w: weak, def: deforming.

[b] *v*: streching; δ: bending.

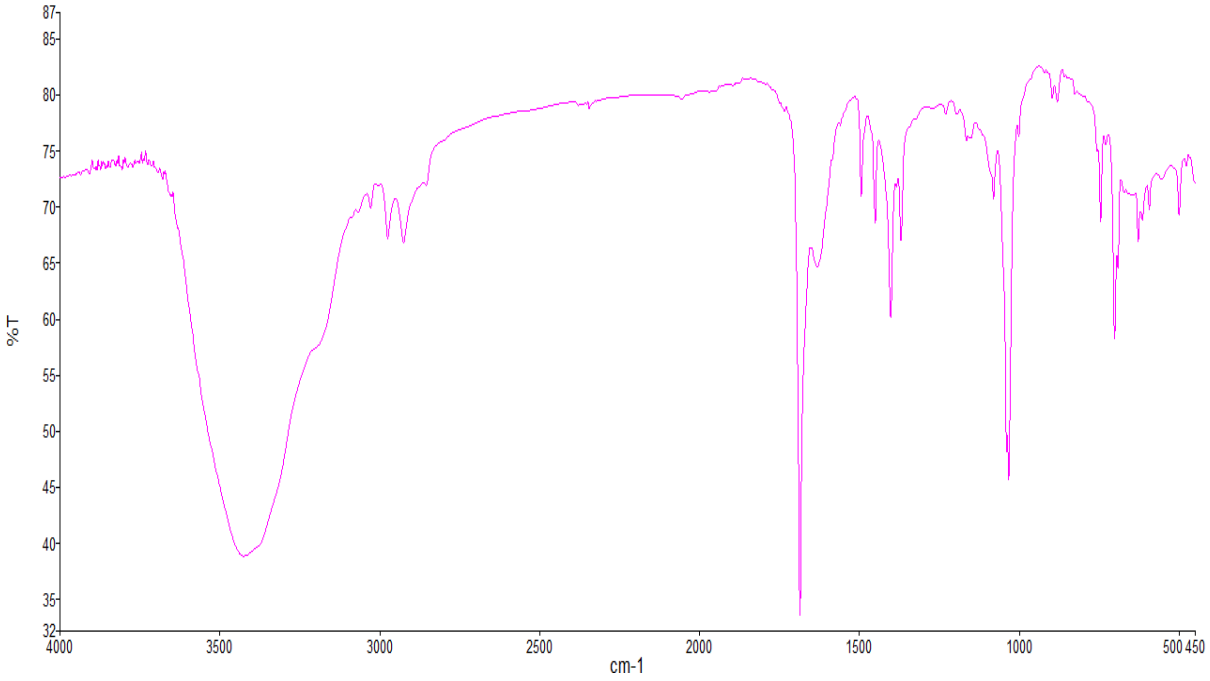

Figure 3.10. Infrared spectra of the pure modafinil.

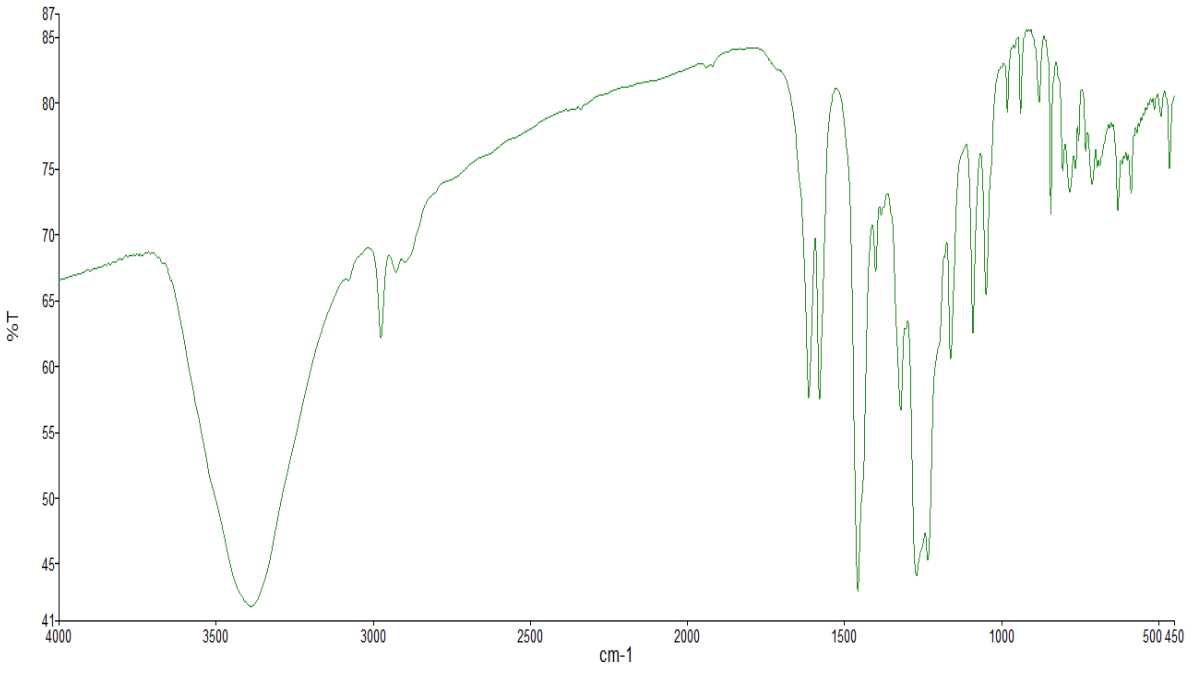

Figure 3.11. Infrared spectra of the quinalizarin reagent.

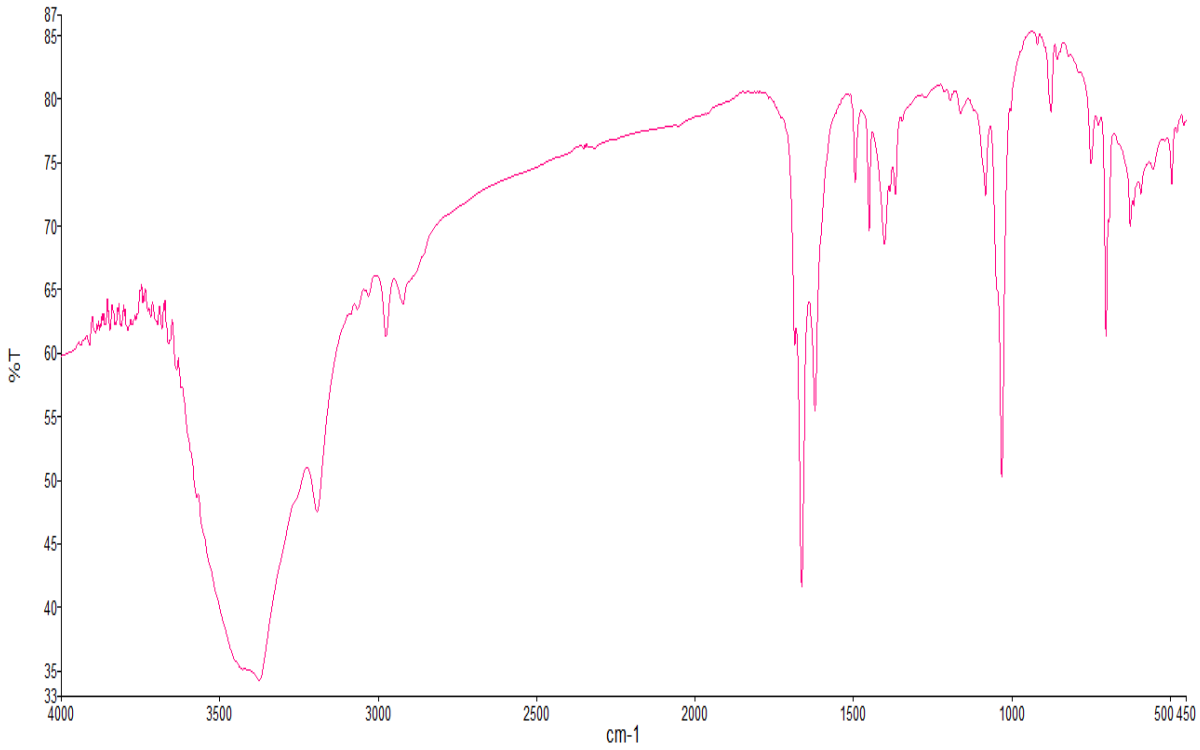

Figure 3.12. Infrared spectra of the modafinil – quinalizarin complex.

4. CONCLUSION

In the present work simple spectrophotometric method was developed this method is proved to be an excellent alternative for the determination of modafinil in pharmaceutical formulations and pure form. It presented acceptable sensitivity and selectivity, allowing the determination of the analyte at levels under those found in the sample. The developed method existing some advantages such as the low cost of equipment (double-beam spectrophotometer) was used with low working cost . The proposed method also have the advantage of being single step reaction and the charge transfer reaction between modafinil and reagent was improved in only one solvent .The method can also be directly applied for modafinil determination in dosage forms containing parabens as the excipients without the requirement of any tedious, time-consuming drug isolation procedure, and it does not require heating, a buffer, or any other solutions. Linear analytical curve was obtained in the range of 20-180$\mu g.mL^{-1}$ and the limits of detection and quantification were 0.35 and 1.2 $\mu g.mL^{-1}$, respectively. Hence, the suggested charge transfer complexation reaction is practical and economical for routine analysis of modafinil in quality control laboratories. In addition to this, the characterization of the synthesized charge transfer complex by infrared spectroscopy has also been described**.**

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