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Simple spectrophotometric methods for quantification of modafinil using 1,2-naphthoquinone-4-sulphonate and 2,4-dinitrophenol as analytical reagents

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Abstract

Two simple visible spectrophotometric methods are developed and validated for the quantification of modafinil using 1,2-naphthoquinone-4-sulphonic acid (NQS method) and 2,4-dinitrophenol (DNP method) as analytical reagents. The NQS method involves the reaction of modafinil with 1,2-naphthoquinone-4-sulphonate in alkaline medium at room conditions to form a yellow colored product exhibiting maximum absorption at 430 nm. DNP method is based on the proton transfer from 2,4-dinitrophenol to modafinil at room conditions and then we have the formation of yellow colored ion-pair complex exhibiting maximum absorption at 475 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 10-100 and 8-60 µg/mL with the detection of limit values of 0.486 and 0.258 µg/mL for NQS method and DNP method, respectively The molar absorptivity and Sandell's sensitivity for both of the methods are reported. The methods were validated in terms of accuracy, precision and robustness. The results

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were satisfactory. The proposed methods were effectively applied to the analysis of the modafinil in their tablet formulations. The recoveries were 99.92% and 99.96% with RSD and 0.863% and 0.722% for NQS and DNS methods, respectively. The assay was not interfered by common excipients.

Keywords: Narcoleptic drug; naphthoquinone-4-sulphonic acid; dinitrophenol; spectrophotometry; analysis.

Introduction

Modafinil (MDF) [1-4], chemically known as 2-[(diphenylmethyl) sulfinyl] acetamide, is a narcoleptic drug that improves wakefulness. The exact mechanism of MDF is not known. It might increase dopamine levels in the brain through binding to the dopamine transporter and reducing dopamine reuptake.

The reported methods for the determination of MDF in biological samples are high performance liquid chromatography [5-10], gas chromatography–mass spectrometry [11] and liquid chromatography-mass spectrometry [12]. Few high performance liquid chromatographic [13-18] and high-performance thin layer chromatographic [19] methods have been described for the quantification of MDF in bulk and pharmaceutical formulations. The above reported chromatographic methods employed sophisticated and expensive 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 methods [5-19] for the quantification of MDF in biological samples, bulk and pharmaceutical formulations are limited.

In underdeveloped and developing countries, UV-Visible spectroscopy is the technique of preference for the precise, accurate and cost-effective determination of pharmaceutical substances. Only one UV spectrophotometric method [13] was reported for the estimation of MDF. It involves absorbance measurement at 236 nm in glacial acetic acid medium. However, the UV spectrophotometric method suffers from major drawbacks. These drawbacks include decreased selectivity due to measurement in UV region, narrow range of linearity, lack of precision and accuracy. To the best of our knowledge, no method has been reported in the literature about the analysis of MDF in bulk and tablet formulations using a visible spectrophotometric method.

In the present work, two simple and sensitive visible spectrophotometric methods (NQS and DNP methods) were developed

and validated for the analysis of MDF with broad linearity, good precision and accuracy. These methods could be applied for the quantitative determination of the MDF in their tablet formulations.

Experimental

Instrumentation

The spectrophotometric measurements were carried out using an Elico double beam model SL 159 digital spectrophotometer with 1 cm matched quartz cells.

Chemicals and reagents

All the chemicals were of analytical reagent grade and were used as received. All the solutions were prepared fresh daily.

1. 0.5% 1,2 Naphthoquinone-4-sulphonic acid (NQS): The solution was prepared by dissolving 500 mg of 1,2 naphthoquinone-4-sulphonic acid (Merck, Mumbai, India) in 100 mL of water.
2. 0.01 N Sodium hydroxide: The solution was prepared by dissolving 40 mg of NaOH (Merck, Mumbai, India) in 100 mL of water.
3. 0.1% 2,4 Dinitrophenol (DNP): The solution was prepared by dissolving 100 mg of DNP (Sdfine-Chem limited, Mumbai, India) in 100 mL of methanol (Merck, Mumbai, India).

Standard MDF solutions

Analytically pure modafinil was obtained as a gift sample from the Orchid Chemicals & Pharmaceuticals Ltd (Chennai, India) and was used as received. For NQS method, the MDF stock standard solution (1 mg/mL) was prepared by dissolving 100 mg of the drug in 20 mL of methanol and then diluted with 100 mL water. Working standard solution containing 500 µg/mL of MDF was prepared by further dilution of the stock standard solution with water. For DNP method, a stock standard solution containing 1 mg/mL of MDF was prepared in methanol. Working standard solution equivalent to 200 µg/mL of MDF was prepared by appropriate dilution of stock standard solution with the same solvent.

General assay procedure

NQS method

Different aliquots (0.2-2.0 mL) of standard MDF solution (500 µg/mL) were accurately transferred into a series of 10 mL calibration flasks. The volume in all the flasks was made up to 2 mL with distilled water. One milliliter of 0.01 N NaOH and 1 mL of 0.5% NQS solution were added to each flask and mixed well. The flasks were kept at room temperature for 10 minutes and diluted to volume with water. The absorbance of the yellow colored chromogen was measured at 430 nm against a *reagent* blank.

DNP method

Different aliquots (0.2-1.5 mL) of standard MDF solution (200 µg/mL) were accurately transferred into a series of 5 mL calibration flasks. The volume in all the flasks was made up to 1.5 mL with methanol. One milliliter of 0.1% DNP solution was added to each flask and diluted to volume with methanol. The content of the flask was mixed well and the absorbance of the yellow colored chromogen was measured at 475 nm against a reagent blank.

In both methods, the calibration curve was constructed by plotting the absorbance against the final concentration of MDF in µg/mL. The amount of MDF in unknown samples was calculated from either the corresponding calibration curve or the corresponding regression equation.

Procedure for the assay of MDF in Pharmaceutical Formulations (Tablets)

The tablet formulation of MDF, Modafil MD 100 (Intas pharmaceuticals, Dehradun, India) labeled to contain 100 mg were purchased from a local pharmacist. Twenty tablets were accurately weighed and finely powdered in a mortar. A portion of tablet powder equivalent to 50 mg of MDF was weighed into a 50 mL beaker; 10 mL of methanol was added and the mixture was shaken for 20 minutes. The mixture was filtered into 50 mL volumetric flask through Whatman No. 1 filter paper. The solution was made up to the mark with distilled water to obtain the stock solution (1 mg/mL). Appropriate volume of the stock solution was then diluted with distilled water to get working standard solution of 500 µg/mL of MDF. Suitable aliquot was subjected to the analysis by NQS method. Another portion of tablet powder equivalent to 50 mg of MDF was accurately weighed into a 50 mL beaker; 30 mL of methanol was added and shaken for 20 minutes. The solution was filtered into a 50 mL volumetric flask through Whatmann No. 1 filter paper and was diluted to the volume with the same solvent to obtain the stock solution (1 mg/mL). The stock solution was diluted aptly with methanol to obtain a working standard solution of 200 µg/mL. Convenient aliquot was subjected to analysis by DNP method. The nominal content of the tablet formulation was calculated either from the previously plotted calibration curves or using regression equation.

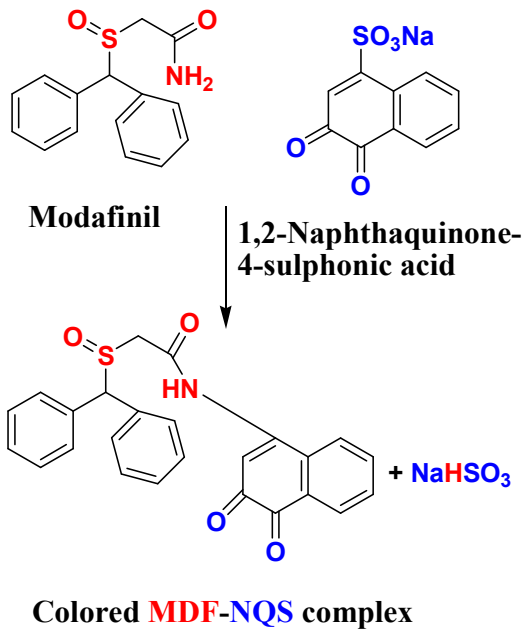
Results and Discussion

Method development

NQS method

The reaction of NQS with amino group of primary and secondary amine derivatives has been reported. These reactions demonstrated that NQS is a valuable chromogenic reagent

in the development of simple spectrophotometric methods for the quantification of many pharmaceutical amines [20-23]. The results obtained in the proposed NQS method are based on the nucleophilic substitution reaction between MDF and NQS in aqueous alkaline medium. The nucleophilic amino group of MDF (due lone electron pair of nitrogen atom) tends to attack the electron deficient center in NQS to form orange yellow colored N-alkyl-amino-naphthoquinone (MDF-NQS complex). The possible reaction scheme is shown in scheme 1.



Scheme 1. Reaction of modafinil with 1,2 naphthoquinone-4-sulphonic acid

The reaction was studied under various conditions of NQS & NaOH concentration and reaction time to determine the optimum conditions for the assay by NQS method.

The effect of the volume of 0.01 N NaOH on the absorbance of the yellow colored MDF-NQS complex was studied in the range of 0.2–2.0 mL. The absorbance increases with the increase in the volume of NaOH upto 1.0 mL. Further addition of NaOH showed decrease in the absorbance. Therefore, 1.0 mL of 0.01 N NaOH was chosen as an optimum value (Figure 1).

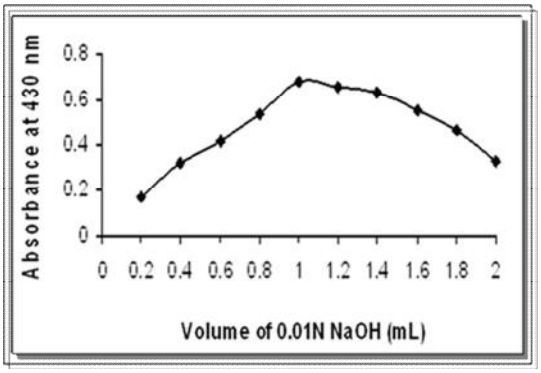


Figure 1. Effect of volume of sodium hydroxide

The effect of the volume of 0.5% NQS on the absorbance of the yellow colored MDF-NQS complex was studied in the range of 0.2–1.4 mL. The absorbance increases with the increase in the volume of NQS and becomes constant at 1.0 mL. Further addition of NQS does not show change in the absorbance. Hence, 1.0 mL of 0.5% NQS was selected as an optimum value (Figure 2).

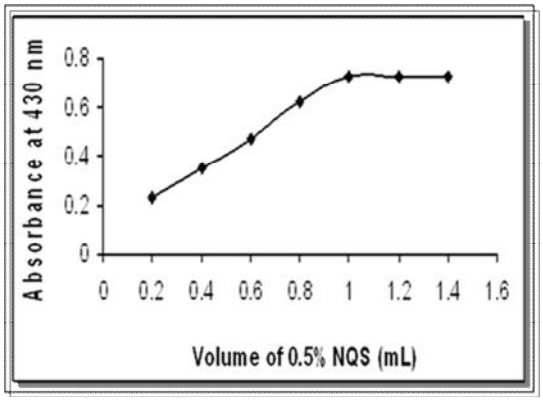


Figure 2. Effect of volume of 1,2 naphthoquinone-4-sulphonic acid

The effect of time on the formation of the yellow colored MDF-NQS complex was also optimized. At room temperature, the color intensity of the product increased by the elapse of time, maximum absorbance was obtained at 10 minutes and remained constant for 30 minutes. A decrease in the absorbance was observed after 30 minutes (Figure 3). The results indicated that the yellow coloured product obtained was stable for 30 minutes.

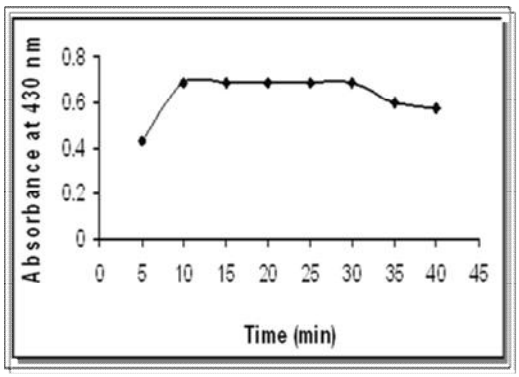
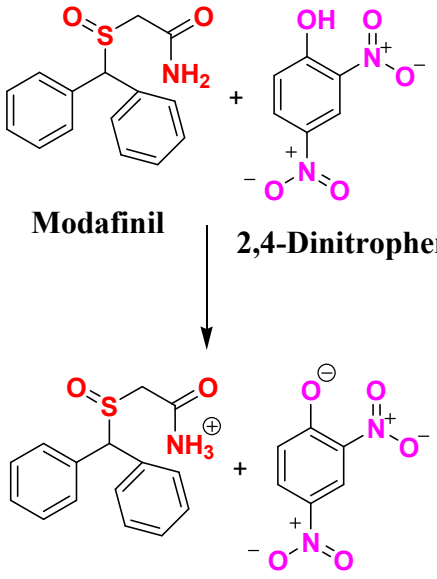


Figure 3. Effect of time

DNP method

The 2,4 dinitrophenol (as Lewis acid) is well known to react with amines (Lewis base) forming stable colored ion pair complex. 2,4 Dinitrophenol, as a chromogenic reagent, has been used for the determination of compounds with primary and secondary amines [24-26]. The results obtained in the proposed DNP method is based on formation of yellow colored ion-pair (MDF-DNP) complex as a result of a proton transfer from hydroxyl group of Lewis acid, 2,4 DNP, to the primary amino group of Lewis base, MDF. The possible reaction scheme is shown in scheme 2.



Scheme 2. Reaction of modafinil with 2,4 dinitrophenol

The parameters like concentration of DNP, reaction time and dilution solvent affecting the color development were studied

to determine the optimum conditions for the assay by DNP method.

The effect of the volume of 0.1% DNP on the absorbance of the yellow colored MDF-DNP complex was studied at the range of 0.5–2.5 mL. The maximum absorbance was observed with 1.0 mL of DNP. Slight decrease in absorbance was observed by further addition of DNP. Hence, 1.0 mL of 0.1% DNP was selected as an optimum value (Figure 4).

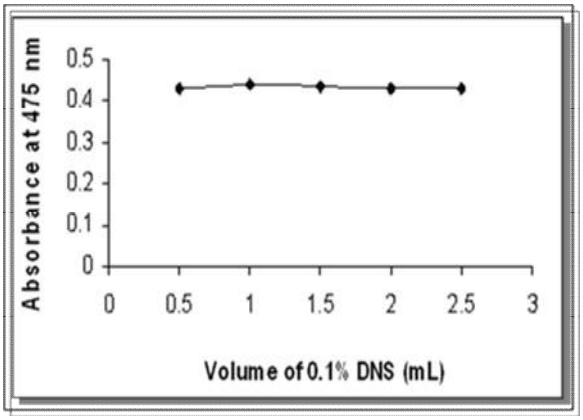


Figure 4. Effect of volume of 2,4 dinitrophenol

The yellow colored MDF-DNP complex was formed instantaneously and the absorbance remained constant at room temperature for about 10 hrs. The absorbance was measured after 2 minutes of mixing with the DNP. The results indicated that the yellow coloured MDF-DNP complex formed was stable for 10 hrs.

In order to choose the proper solvent for dilution, different solvents (methanol, dichloromethane, chloroform, isopropanol and acetonitrile) were tested. The highest absorbance values were obtained when methanol was used as a diluting solvent. In methanol medium, the absorbance values were found to be stable for 10 hrs. Hence methanol was chosen as the dilution solvent.

Association constant and the free energy changes of the complexes

The association constant of MDF-NQS and MDF-DNA complexes were determined by employing the Benesi–Hildebrand method [27], besides the association constant was calculated by using the following equation:

$$[Ao]/A\lambda = 1/\varepsilon + (1/Kc.\varepsilon).1/[Do]$$

Where **[Do]** = Concentration of the drug, **[Ao]** = Concentration of the reagent, **Aλ** = Absorbance of the MDF-NQS and MDF-DNA complexes at 430 and 475 nm, respectively, **ε** = Molar absorptivity of the MDF-NQS and MDF-DNA complexes at 430 and 475 nm, respectively. **Kc** = Association constant of the complex.

The ***ΔG°*** (the standard free energy of complexation) and the association constant **Kc** are related by the following equation [28]:

$$\Delta G° = -2.303RT\log Kc$$

Where ***ΔG°*** = Free energy change of the complex, ***R*** = Gas constant (1.987 cal mol^{-1} $degree^{-1}$), ***T*** = Temperature

in Kelvin, ***K*** = Association constant (L mol^{-1}) of the drug-reagent complex.

The results are summarized in the Table 1. The negative values of the standard free energy indicated that the complexes are stable and started to form spontaneously.

Analytical performance of the proposed NQS and DNP methods

Linearity and range

The relation between the absorbance and final concentration of MDF was found to be linear over the concentration range of 10-100 µg/mL (NQS method) and 8-60 µg/mL (DNP method). The results of the linear regression analysis of calibration data are summarized in Table 1.

Sensetivity

According to the ICH guidelines [29], the sensitivity parameters like molar absorptivity, Sandell's sensitivity, limits of detection (LOD) and limits of quantification (LOQ) were calculated for both of the methods. The results (Table 1 indicate the sufficient sensitivity of the methods.

Precision

The repeatability (intra-day precision) of the proposed methods was determined by replicate analysis (n=5) of standard solutions at three concentration levels (NQS method-10, 50 and 100 µg/mL: DNP method-8, 32 and 60 µg/mL). The intermediate precision (inter-day precision) was conducted by repeating the analysis over a period of three consecutive days.

Table 1. Thermodynamic studies, linearity and Sensitivity of the proposed methods

Parameter	Method	
	NQS	**DNP**
Beer's law limit (µg/mL)	10-100	8-60
Regression equation (y = mc+x)*	y = 0.0074c + 0.0133	y = 0.0114c - 0.0048
Slope (m)	0.0074	0.0114
Intercept (x)	0.0133	-0.0048
Regression coefficient (r^2)	0.9991	0.9993
Molar Absorbtivity (L $mole^{-1}$ cm^{-1})	2.542 x 10^4	3.041 x 10^4
Sandell's sensitivity	0.010750	0.008988

(µg cm^{-2}/0.001 Absorbance unit)		
LOD (µg/mL)	0.486	0.258
LOQ (µg/mL)	1.620	0.860
Association constant (L $mole^{-1}$)	5.206 x 10^3	2.067 x 10^4
Free energy change (kJ mol^{-1})	-4.543 x 10^3	-5.391 x 10^3

*y = Absorbance; c = Concentration of MDF in µg/mL

The precision of the methods was expressed as standard deviation (SD) and percentage relative standard deviation (% RSD). The results are summarized in Table 2. The SD and %RSD obtained by both methods are found to be in the acceptable range. Therefore, it can be considered to be satisfactory.

Accuracy

The accuracy of the proposed methods was established by performing intra-day and inter-day assays by determining the concentration of MDF at three different concentration levels (NQS method-10, 50 and 100 µg/mL: DNP method-8, 32 and 60 µg/mL) within 1 day and on 3 consecutive days, respectively. The accuracy of the methods is expressed as percentage recoveries and percentage error (Table 2). The results obtained by both methods are found to be in the acceptable range. Therefore, we can say that it can be considered as satisfactory.

Table 2. Accuracy and Precision of the proposed methods

Type of assay	Method	MDF (µg/mL)		SD*	% RSD	% Recovery	% Error
		Taken	Found				
Intra-day	NQS	10	9.96	0.093	0.933	99.60	0.04
		50	50.06	0.368	0.735	100.12	0.12
		100	99.92	0.893	0.893	99.92	0.08
	DNP	8	7.95	0.039	0.490	99.37	0.63
		32	32.03	0.185	0.577	100.09	0.09
		60	59.94	0.423	0.705	99.90	0.10
		10	10.02	0.101	1.007	100.20	0.20

	NQS	50	49.98	0.442	0.884	99.96	0.04
Inter-day		100	99.94	0.943	0.943	99.94	0.06
		8	8.02	0.044	0.548	100.25	0.25
	DNP	32	31.94	0.265	0.829	99.81	0.19
		60	60.04	0.515	0.857	100.07	0.07

*for five determinations

In addition, accuracy and validity of the proposed methods were determined by standard addition technique. The preanalyzed samples were spiked with additional 50, 100 and 150 % of the MDF and the mixtures were once again analyzed by the proposed methods. The accuracy of the methods was evaluated by percentage recovery of the MDF. The average recovery and percentage standard deviation values (Table 3) of the methods lying in the acceptable range show that the methods are accurate and free from interference of excipients.

Table 3. Results of standard addition technique of the proposed methods

Method	**Concentration of MDF (mg)**			**SD***	**% RSD**	**% Recovery**
	Tablet	**Spiked**	**Found**			
	100	50	149.95	1.104	0.736	99.96
NQS	100	100	200.15	0.963	0.481	100.07
	100	150	249.93	1.008	0.403	99.97
	100	50	149.95	0.983	0.655	99.96
DNP	100	100	200.06	0.764	0.381	100.03
	100	150	249.92	0.966	0.386	99.97

*for five determinations

Robustness

The robustness of the proposed methods was checked for each operational parameter and investigated. The operational parameters were:

NQS method

- Volume of 0.01N NaOH: 1 ± 0.1 mL
- Volume of 0.5% NQS: 1 ± 0.1 mL
- **Reaction time: 10 ± 2 Minute**

DNP method

- Volume of 0.1% DNP: 1 ± 0.1 mL

The robustness of the methods was assessed by analyzing the MDF at two different concentration levels (NQS method - 10 and 100 µg/mL: DNP method – 8 and 60 µg/mL). The percent recovery and %RSD of the methods (Table 4) were found to be satisfactory, indicating that the methods are robust.

Application of the proposed methods to analysis of MDF in tablet formulations

It is obvious from the above-mentioned results that the proposed methods gave satisfactory results with MDF in bulk. Therefore, MDF tablet formulations were subjected to the analysis of their MDF contents by the proposed methods. The percent recovery and %RSD (Table 5) clearly showed no interference of any excipients of formulation, thus proving accuracy & precision in the quantification of MDF by both methods.

Table 4. Robustness of the proposed method

Experimental variable	Concentration of MDF					
	10* or 8** µg/mL			100* or 60** µg/mL		
	Found ± SD (n=3)	% Recovery	% RSD	Found ± SD (n=3)	% Recovery	% RSD
NQS method						
Volume of 0.01N NaOH (1 ± 0.1 mL)	9.96 ± 0.086	99.60	0.863	100.05 ± 0.821	100.05	0.820
Volume of 0.5% NQS (1 ± 0.1 mL)	10.06 ± 0.108	100.60	1.073	99.92 ± 0.714	99.92	0.714

Reaction time(10 ± 2 minute)	9.97 ± 0.098	99.70	0.982	99.90 ± 0.798	99.90	0.798
DNS method						
Volume of 0.1% DNP (1 ± 0.1 mL)	8.04 ± 0.033	100.50	0.41	59.93 ± 0.322	99.88	0.537

*Concentration of MDF taken in NQS method
**Concentration of MDF taken in DNA method

Table 5. Results of analysis of MDF in tablet formulations

Method	Concentration of MDF (mg)		% RSD	% Re-covery
	Tablet	Found ± SD (n=5)		
NQS	100	99.92 ± 0.863	0.863	99.92
DNP	100	99.96 ± 0.722	0.722	99.96

*for five determinations

Conclusion

The present study described the successful evaluation of NQS and DNP as analytical reagents in the development of simple and rapid visible spectrophotometric methods for the analysis of MDF in its tablet formulations. The methods described herein have many advantages. The proposed methods don't require any expensive sophisticated apparatus. The methods are simple, rapid and robust and have high precision and accuracy. The NQS and DNP are inexpensive reagents and are available in any analytical laboratory. Hence, these methods are valuable for its routine application in quality control laboratories for the analysis of MDF.

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