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A Review on Analytical Methods for Estimation of Modafinil

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ABSTRACT

Modafinil is a wakefulness-promoting agent (or eugeroic) used for treatment of disorders such as narcolepsy, shift work sleep disorder, and excessive daytime sleepiness associated with obstructive sleep apnea. It is sold under the brand names Alertec, Modavigil, and Provigil. Various analytical methods used for the estimation of Modafinil have been reviewed in this paper. These include Ultraviolet Spectrophotometry, RP-HPLC, Stability indicating RP-HPLC, HPTLC, Capillary zone electrophoresis, simultaneous determination by HPLC, LC-MS/MS, Liquid –liquid extraction, HPLC , bidimensional HPLC, GC-MS and Solid-phase extraction followed by HPLC in rat serum and urine to determine the amount of Modafinil in bulk drug, pharmaceutical dosage form and biological fluids. These analytical methods can be used for qualitative and quantitative estimation of Modafinil in bulk, formulation and biological fluids.

Keywords: Modafinil, bulk drug, dosage form, biological fluids, analytical methods, estimation.

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INTRODUCTION

Modafinil is extensively used as wakefulness promoting agent for oral administration. Its IUPAC name is [2-(1, 1-diphenyl methyl sulfinyl) acetamide]. Its empirical formula and molecular weight is $C_{15}H_{15}NO_2S$ and 273.35 gm/mol respectively. It is a white to off-white, crystalline powder. It is slightly soluble or practically insoluble in water, slightly soluble in ethanol and sparingly soluble in methanol. Its melting point is 164-166ºC. This compound belongs to the class of organic compounds known as diphenylmethanes. These are compounds containing a diphenylmethane moiety, which consists of methane wherein two hydrogen atoms are replaced by two phenyl groups[1].

Modafinil is a stimulant drug marketed as a 'wakefulness promoting agent' and is one of the stimulants used in the treatment of narcolepsy. Narcolepsy is caused by dysfunction of a family of wakefulness-promoting and sleep-suppressing peptides, the orexins, whose neurons are activated by modafinil. The prexin neuron activation is associated with psychoactivation and euphoria. It is indicated to improve wakefulness in patients with excessive daytime sleepiness (EDS) associated with narcolepsy[1].

The exact mechanism of action is unclear, although *in vitro* studies have shown it to inhibit the reuptake of dopamine by binding to the dopamine reuptake pump, and lead to an increase in extracellular dopamine. Modafinil activates glutamatergic circuits while inhibiting GABA. Modafinil is thought to have less potential for abuse than other stimulants due to the absence of any significant euphoric or pleasurable effects. It is possible that modafinil acts by a synergistic combination of mechanisms including direct inhibition of dopamine reuptake, indirect inhibition of noradrenalin reuptake in the VLPO and orexin activation. Modafinil has partial alpha 1B-adrenergic agonist effects by directly stimulating the receptors[1].

The optical enantiomers of modafinil have similar pharmacological actions in animals. Two major metabolites of modafinil, modafinil acid and modafinil sulfone, do not appear to contribute to the CNS-activating properties of modafinil[2].

Absorption of modafinil is rapid following by oral administration. Tmax is 2 to 4 hours. Food delays T max approximately 1 hour. Apparent volume of distribution (Vd) is 0.9 L/kg and protein binding is approximately 60%. Hepatic metabolism of modafinil is take place by CYP-450 3A4 enzyme and converts into modafinil acid.

The major route of elimination is metabolism (~90%), primarily by the liver, with subsequent renal elimination of the metabolites. The half-life is approximately 15 hours[3].

Side effects of Modafinil are Headache, nervousness, insomnia, agitation, confusion, personality disorders, tremor, anxiety; GI disturbances, hypertension, palpitations, tachycardia, angioedema, psychosis, depression, mania, abnormal LFTs. Stevens- Johnsons syndrome, erythema multiforme, toxic epidermal necrolysis. Special Precautions are taken in case of history of psychosis, depression, mania. Discontinue use if there is any sign of rash of hypersensitivity reaction. Increase monitoring in patients with hypertension[3].

ANALYTICAL METHODS FOR MODAFINIL

Various analytical methods have been reported for the estimation of Modafinil in bulk drug and pharmaceutical dosage form as well as in biological fluids.

ESTIMATION OF MODAFINIL IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM

Spectrophotometric Methods:

C.B. Sekaran et al. developed 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 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[4].

Rashmi, N. G.; Chandan, R. S.; Gurupadayya, B. M.; Srujana, S.; Raagaleena, V. developed two simple, extraction free Spectrophotometric methods for the quantitative determination of modafinil in bulk drugs and pharmaceutical formulations. The method I is based on the oxidation of 2,4-dinitrophenyl hydrazine (2,4- DNP) and coupling of the oxidized product with drugs to gave intensely colored chromogen. The condensed product of modafinil shows linearity in the

concentration range of 2.0-7.0 mg/mL with λmax 476 nm. The method II is based on a condensation reaction between modafinil and acidic solution of p-dimethyl amino cinnam aldehyde (PDAC) to form instant reddish brown colored product showed linearity in the concentration range of 6.0-14.0 mg/mL with λmax 443 nm. Recovery studies for modafinil were found to be 99.9% and 99.6%. The %RSD values were found to be 0.6% and 1.6% for method I and II respectively. No interference is observed from excipients and the proposed method was statistically validated[5].

Pawan Kumar Basniwal developed three new validated spectrophotometric methods. For determination of modafinil in tablet formulation. In this study, three new UV spectrophotometric methods viz. linear regression equation (LRE), standard absorptivity (SA) and first order derivative (FOD) method were developed and validated for determination of modafinil in tablet form. The Beer-Lambert's law was obeyed as linear in the range of 10-50 µg/mL and all the methods were validated for linearity, accuracy, precision and robustness. These methods were successfully applied for assay of modafinil drug content in tablets in the range of 100.20-100.42%, 100.11-100.58% and 100.25-100.34%, respectively with acceptable standard deviation (less than two) for all the methods. The validated spectrophotometric methods may be successfully applied for assay, dissolution studies, bio-equivalence studies as well as routine analysis in pharmaceutical industries[6].

Chromatographic Methods:

Chromatographic methods like RP-HPLC and Stability indicating RP-HPLC, HPTLC have been reported for the estimation of Modafinil in bulk drug and pharmaceutical dosage form.

HPLC Methods

V.Venkatesh et al. developed two methods for determination of Modafinil in solid dosage form. The first method was based on UV-Spectrophotometric determination of the drug. It involves absorbance measurement at 236nm (l max of Modafinil) in Glacial acetic acid. Calibration curve was linear with the correlation coefficient was 0.999 over a concentration range of 5 to 30µg/ml for the drug. The second method was based on HPLC separation of the drug in reverse phase mode using Hypersil ODS C18 column (250 X 4.6mm, 5mm). The mobile phase constituted of Acetonitrile: Glacial acetic acid (80:20) and the flow rate 1.0 ml/ min. Detection was performed at 236nm. Separation was completed within 5 min. Calibration curve was linear with the correlation coefficient was 0.999 over a concentration range of 50 to 300µg/ml for the drug. The relative standard deviation (R.S.D) was found <2.0% For U.V. Spectrophotometry and HPLC methods.

Both these methods have been successively applied to solid dosage pharmaceutical formulation. The present methods were validated according to ICH guidelines[7].

Y. Indira Muzib et al. developed a simple, accurate and sensitive RP-HPLC method and validated for the estimation of Modafinil in pure drug and it's tablet dosage form. A reverse phase high performance liquid chromatographic method was performed by using Agilent,XDBC18 column (100 mmX 4.6 mmX5µ particle size) with UV detection at 225 nm. An isocratic mobile phase consisting of Potassium dihydrogen Phosphate Acetonitrile: 50:50 (v/v) at a flow rate of 1ml/min. The retention time for Modafinil was 4.32 min. The method was linear in the concentration range of 12.5-75 µg/ml of Modafinil with the correlation coefficient of 0.999. The method was validated for linearity, accuracy, precision, limit of detection, limit of quantification, robustness and ruggedness. Recovery of Modafinil was found to be 99% to 101%.The developed reverse phase high performance liquid chromatographic method was simple, sensitive, precise and accurate and the method was found suitable for estimating in tablet dosage form[8].

Shanmugasundaram et al. developed a simple, precise, rapid, and accurate RP-HPLC method was developed for the estimation of modafinil in bulk and pharmaceutical dosage forms. Xterra RP 18 (250 mm × 4.6 mm, 5 µ particle size) with a mobile phase consisting of methanol: water 70:30 V/V was used. The flow rate 1.0 ml/min and the effluents were monitored at 260 nm. The retention time and recovery time was 12 minutes. The detector response was linear in the concentration of 10-50 µg/ml. The respective linear regression equation being Y=452.1x+65237. The limit of detection and limit of quantification were 4.547 and 1.377 mcg, respectively. The method was validated by determining its accuracy, precision, and system suitability according to ICH guidelines[9].

Bhimanadhuni et al. developed a reverse phase high performance liquid chromatographic method was developed for the determination of Modafinil in bulk and dosage form. The separation was effective on a Hypersil ODS C18 column (250 mm x 4.6 mm; 5µ) using a mobile phase mixture of Buffer: Acetonitrile in a ratio of 55:45 (v/v) at a flow rate of 1.0ml/min. The detection was made at 220nm. The retention time of modafinil was found to be 4.80±0.06 min. Calibration curve was linear over the concentration range of 20-120µg/ml of modafinil. The proposed method was validated as per the ICH guidelines. The method was accurate, precise, specific and rapid and thus found to be suitable for the quantitative analysis of modafinil in the bulk and dosage form[10].

Burla Sunitha Venkata Seshamamba, Peruri Veera Venkata Satyanarayana, Chandra Bala Sekaran, developed a stability indicating HPLC method was developed and validated for modafinil quantification in bulk and tablet dosage forms. Aligent zorebax SB C18 analytical column (250

mm x 4.6 mm, 5 µm particle size) was used with mobile phase consisting of 0.1 M sodium dihydrogen phosphate and methanol in ratio 60:40 (v/v), flow-rate 1.0 ml/min, UV-detection at 230 nm and controlled temperature at 30°C. The linearity was found in the concentration range of 5-150 µg/ml. The method was validated as per the ICH guidelines. The drug was exposed to acidic, basic, oxidation, photo degradation and dry heat conditions. As the developed method can efficiently separate the modafinil from its degradation products, it can be employed as stability-indicating method[11].

Vilas Chaudhary and Milind Ubale developed an isocratic reversed phase stability-indicating high-performance liquid chromatographic (HPLC) assay method and validated for quantitative determination of Modafinil hydrochloride in bulk drugs. An isocratic, reversed phase HPLC method was developed to separate the drug from the degradation products, using an Thermo Hypersil C18 (250 x 4.6) mm, 5µ column and the mobile phase containing 2.0gm potassium dihydrogen phosphate and 1.0 gm 1-octaneSulfonic acid salt in 1000ml water filter and mixed. Prepare a homogenous mixture of buffer, and Acetonitrile (35:65, v/v). The detection was carried out at wavelength 210 nm. The developed method was validated with respect to linearity, accuracy (recovery), precision, system suitability, selectivity, robustness prove the stability indicating ability of the method[12].

HPTLC Methods

Gaurang P. Pandya, Dr. Hitendra S. Joshi developed a simple, selective, precise and stability-indicating high-performance thin layer chromatographic method for analysis of Modafinil, both as the bulk drug and in a tablet formulation has been developed and validated. Aluminum foil TLC plates precoated with silica gel 60F 254 were used as stationary phase ethyl acetate, acetone and methanol in the volume ratio of (7:2:1 v/v) respectively as mobile phase. A compact band (Rf 0.42±0.02) was obtained for modafinil. Densitometric analysis was performed in absorbance mode at 232 nm. Linear regression analysis revealed a good linear relationship (r2=0.9995) between peak area and concentration in the range of 80-320 ng /spot. The method was validated for precision, recovery, and robustness. The limits of detection and quantitation were 15 and 50 ng/spot, respectively. Modafinil was subjected to acid and alkaline hydrolysis, oxidation, photochemical and thermal degradation and underwent degradation under all these conditions. Statistical analysis proved the method enables repeatable, selective, and accurate analysis of the drug. It can be used for identification and quantitative analysis of Modafinil in the bulk drug and in tablet formulations[13].

Capillary zone electrophoresis

Al Azzam, Khaldun M.; Saad, Bahruddin; Aboul-Enein, Hassan Y.; Elbashir, Abdalla A. have developed and validated simple, sensitive, and cost effective capillary zone electrophoresis (CZE) method for the determination of the novel wake promoting agent, modafinil in pharmaceutical formulations. The CZE separation was performed using 50 mm i.d. × 56 cm fused silica capillary under the following conditions: capillary temp. 25°C, applied voltage, 25 kV; 20 mM H3PO4 M tris running buffer (pH 9.0); detection wavelength, 225 nm. Phenobarbital was used as the internal standard. The method was validated and showed not only good precision and accuracy but also good robustness. The calibration was linear from 5 to 250 mg/mL. The accuracy values ranged from 101.6% to 105.3%. The good accuracy values obtained indicate the potential of this method for the determination of the analyte in pharmaceutical formulations. The LOD and LOQ were 1.2 and 3.5 mg/mL-1, respectively. The method has been successfully applied to the determination of modafinil in pharmaceutical tablet formulations. Excipients present in the tablets and degraded products from different stress conditions did not interfere in the assay[14].

ESTIMATION OF MODAFINIL IN BIOLOGICAL FLUIDS

Various methods have been reported for the estimation of Modafinil in biological fluids like plasma, serum, urine and saliva.

Plasma:

HPLC

G Moachon, D Matinier developed sensitive and selective high-performance liquid chromatographic (HPLC) method for the simultaneous quantitation of modafinil and its acid metabolite in human plasma has been developed. The method is based on a liquid-liquid extraction followed by isocratic reversed-phase HPLC with ultraviolet absorbance detection at 236 nm. The eluent used was acetonitrile-water-acetic acid (150:420:12, v/v/v). The run time was 45 in. The method provided a detection limit of 0.04 mg/l for modafinil and the acid metabolite, a quantitation limit of 0.13 mg/l for modafinil and 0.14 mg/l for the acid metabolite. A good linear relationship was obtained in the concentration range studied (0.1-20 mg/l) for both compounds and the method was sufficiently accurate and precise to support clinical pharmacokinetic studies. It is the first described method for determination of modafinil and its acid metabolite in plasma[15].

Liquid–liquid extraction and high-performance liquid chromatography

Gorman et.al. developed modafinil, modafinil acid and modafinil sulfone in human plasma utilizing liquid–liquid extraction and high-performance liquid chromatography. An assay was developed to determine concentrations of modafinil (*dl*-2-[(diphenylmethyl)sulfinyl]acetamide; Provigil®) and its two major circulating metabolites, modafinil acid and modafinil sulfone, in

human plasma. The assay utilized liquid–liquid extraction of the analytes and an internal standard, (phenylthio) acetic acid, from plasma into a mixture of hexane–dichloromethane–glacial acetic acid (55:45:2, v/v). The analytes were resolved isocratically on a narrow-bore phenyl column at a mobile phase flow-rate of 0.3 ml/min and were monitored by UV detection at 235 nm. The method reported herein reduces the required sample volume of previously reported methods from 1.00 to 0.200 ml of plasma while lowering the limit of quantification (LOQ). The linear range of the assay was from 0.100 to 20.0 μg/ml for each of the three compounds[16].

Bidimensional HPLC

Quezia B. Cass , Túlio Ferreira Galatti have developed a method for determination of the plasma levels of modafinil enantiomers, (±)-modafinic acid and modafinil sulphone by direct human plasma injection and bidimensional achiral–chiral chromatography Coupled-column separation using restricted access media as the first dimension in order to exclude macromolecules and retain micromolecules has been successfully used for a number of biological fluids. This paper describes the first method developed and validated for the analysis in a single run of the enantiomers of modafinil and its two major metabolites. The method was developed using a bidimensional HPLC system by coupling a restricted access medium (RAM) bovine serum albumin (BSA) column (1.0 cm × 0.46 cm i.d.) to an amylose tris[(S)-1-phenylethylcarbamate] chiral column. The method was fully validated and showed good linearity, precision, accuracy, sensitivity and selectivity, allowing it to be used for pharmacokinetic studies. The quality of the performance of both columns was maintained with over 280 plasma injections of 100 μl[17].

LC-MS/MS Method

Pankaj et al. developed a rapid, selective and sensitive high performance liquid chromatography–tandem mass spectrometry method (LC-MS/MS) was developed and validated for the estimation of modafinil in human plasma. Modafinil and the internal standard (ISTD), Modafinil-D5, were extracted from plasma samples using solid phase extraction with Agilent® Bond Elut Plexa cartridges. Chromatographic separation was performed on a Ascentis® C18 column (150mm×4.6mm, 5μm) using methanol:2mM ammonium acetate: glacial acetic acid (35:65:0.1% v/v/v) as the mobile phase at a flow rate of 1.0 mL/min. Detection of modafinil and modafinil-D5 was achieved by tandem mass spectrometry with an electrospray ionization (ESI) interface in positive ion mode. The calibration curves were linear over the range of 30.8 to 8022.1 ng/mL. The method has a lower limit of quantitation (LLOQ) of 30.8 ng/mL and the limit of detection (LOD) achieved of 1 ng/mL for modafinil, based on a signal to noise ratio of 10. The intra- and inter-day

precisions were within 3.1%, while the accuracy was within ±3.3% of nominal values. No matrix effect was observed in this method[18].

Urine:

GC/MS

Tseng et al., developed a method for detection of modafinil in human urine by gas chromatography–mass spectrometry. The main purpose of this study was to detect and quantify modafinil in human urine by gas chromatography–mass spectrometry (GC–MS). Urinary samples were collected from three healthy male volunteers following oral administration of a clinical dose (100 mg) of modafinil (Provigil®). Urine specimens were extracted with *t*-butylmethyl ether (TBME) prior to GC–MS analysis. The results demonstrate that the chromatographic characteristics and the mass spectrum of the unchanged parent drug extracted from urine samples were identical to that obtained from the authentic standard. The times for the unchanged modafinil to reach peak concentration in the urine of the three volunteers were at 2 h (6.14 μg/mL), 4 h (9.93 μg/mL) and 8 h (3.58 μg/mL), respectively. Total clearance occurred in approximately 48–72 h with 2–5% eliminated through urine as unchanged modafinil. The present study demonstrates that modafinil is detectable in the absence of hydrolysis and derivatization steps[19].

Plasma and urine:

HPLC

Schwertner et.al, developed a high-performance liquid-chromatographic procedure (HPLC) for the quantitative analysis of modafinil in plasma and urine. (Phenylthio) acetic acid was used as an internal standard for the analysis of both plasma and urine. Modafinil was extracted from urine and plasma with ethyl acetate and ethyl acetate–acetic acid (100:1, v/v), respectively, and analyzed on a C18 reverse phase column with methanol–water–acetic acid (500:500:1, v/v) as the mobile phase. Recoveries from urine and plasma were 80.0 and 98.9%, respectively and the limit of quantitation was 0.1 μg/mL at 233 nm. Forty-eight 2-h post-dose urine samples from sham controls and from individuals taking 200 or 400 mg of modafinil were analyzed without knowledge of drug administration. All 16-placebo urine samples and all 32 2-h post-dose urine samples were correctly classified. The analytical procedure is accurate and reproducible and can be used for therapeutic drug monitoring, pharmacokinetic studies, and drug abuse screening[20].

Rat serum and urine:

Solid-phase extraction followed by HPLC.

R. Nageswara Rao, Dhananjay D. Shinde. have developed enantioselective separation and determination of adrafinil and modafinil on Chiralcel OJ-H column in rat serum and urine using

solid-phase extraction followed by HPLC.A simple and rapid normal-phase HPLC method for enantiospecific separation of a psychostimulant, adrafinil (ADL), and its metabolite modafinil (MDL) in rat serum and urine was developed. The separation was accomplished on a normal-phase polysaccharide stationary phase Chiralcel OJ-H using *n*-hexane–ethanol (62:38 v/v) as a mobile phase at a flow rate of 1.0 mL/min. Detection was carried out at 225 nm using a photo diode array (PDA) detector. The elution order of the enantiomers was determined by a polarimeter connected in series with the PDA. ADL and its metabolite were recovered from rat serum and urine by solid phase extraction using Oasis HLB cartridges and the mean recoveries were ≥80%. The enantiomers were eluted within 15 min without any interference from endogenous substances. The calibration curves were linear (r^2 > 0.998) in the concentration range of 1.20–500 μg/mL for ADL and MDL. The assay was specific, accurate, precise and reproducible (intra- and inter-day precisions RSDs <7.2%). ADL in rat serum was stable over three freeze–thaw cycles at ambient temperature for 4 h. The method was successfully applied to pharmacokinetic studies of adrafinil after an oral administration to rats[21].

CONCLUSION

Various analytical methods have been reported for the estimation of Modafinil in pharmaceutical formulations and biological fluids. UV Spectrophotometry, HPLC, Stability indicating RP-HPLC, HPTLC, and Capillary zone electrophoresis are the methods for estimation of Modafinil in bulk drug and pharmaceutical dosage form and HPLC**,** simultaneous determination by HPLC, LC-MS/MS Method, Liquid–liquid extraction and high-performance liquid chromatography, bidimensional HPLC, GC/MS and solid phase extraction followed by HPLC are the methods for estimation of Modafinil in biological fluids. However, the most widely used method for the estimation of Modafinil in bulk drug and pharmaceutical dosage form is HPLC method.

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