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B. Ramachandra

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A Critical Review of Properties of Modafinil and Analytical, Bio Analytical Methods for Its Determination

B. Ramachandra^{1,*}

¹Government Degree College, Rajampeta-516115, Kadapa Dist, Andhra Pradesh, India.

*Correspondence to: B. Ramachandra, Government Degree College, Rajampeta-516115, Kadapa Dist, Andhra Pradesh, India Phone No: +91-9492753765 E-mail: bramachandra84@gmail.com

Abstract

Modafinil is a synthetic molecule used for the treatment of narcolepsy. Narcolepsy is a neurological disorder, due to which people experience frequent excessive daytime sleepiness. Nevertheless, there are some concerns about modafinil quality control. The modafinil enantiomers are both biologically active. However, it has been reported that the pharmacological properties of the both enantiomers are different and that the S-enantiomer is eliminated three times faster than the R-enantiomer. Therefore, most of the pharmaceutical companies have shifted to produce of armodafinil (R- enantiomer) instead of the racemate. This article discusses about the critical review of the literature, the impact of the use of modafinil in the treatment of narcolepsy patients and other diseases, its physicochemical properties, toxicological properties, synthetic methods, analytical and bio analytical methods, and challenges that remain in order to ensure the quality. This article mainly focused on review of process related impurities, enantiomeric separation, metabolites of modafinil in various biofluids and pharmaceutical formulations using HPLC, LC-MS, GC-MS, CE, HPTLC and spectrophotometric methods.

Key words

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Modafinil, Armodafinil, Narcolepsy, CNS stimulant, Enantioseparation, Process related impurities, Metabolites

1.0 Introduction

Narcolepsy is a neurological disorder, due to which people experience persistent excessive daytime sleepiness (EDS). A person with narcolepsy may fall in sleep at inappropriate times and get tired throughout the day. Daytime naps may occur many times in a day and drowsiness may continue for prolonged periods. In addition to that night time sleep may be disturbed due to frequent awakenings [1]. It is especially distressing and potentially dangerous disorder that impairs the quality of life. Other symptoms of narcolepsy include hypnagogic hallucinations, cataplexy and sleep paralysis. It affects approximately 0.06% of the population in North America and Western Europe [2].

Central nervous system (CNS) stimulants are mainly used for treatment of narcolepsy. Although CNS stimulants such as methylphenidate, amphetamine are used to narcolepsy as well as attention deficit/hyperactivity disorders (ADHD), they are not effective to control EDS in most of the narcoleptic patients [3,4]. However, these compounds have potential risk of dependence and also tolerance. As a result, a novel non stimulant for treatment of ADHD and narcolepsy has been focus of new researches. In general drowsiness is usually treated by stimulants such as amphetamine, methamphetamine, methylphenidate, dextroamphetamine, atomoxetine, codeine and selegiline [5]. One such drug is modafinil, a unique psycho-stimulant α 1-adrenoreceptor agonist. It was discovered at French pharmaceutical company by the Laboratoire L. Lafon in 1994. Modafinil is marketed by Cephalon Inc., as Provigil® who originally leased the rights from Lafon and finally purchased the company in 2001[6,7,8]. Because of its wakening properties, absence of tolerance-producing effects and low toxicity, it could be used by armed forces in sustained or continuous operations involving partial or total

sleep deprivation [9]. It has been approved by the Food and Drug Administration (FDA) for treatment of narcolepsy in 1998 and in 2003 for shift work sleep disorders and obstructive sleep apnea/hypopnea [10].

This article discusses about the critical review of the literature, the impact of the use of modafinil in the treatment of narcolepsy patients and other diseases, its physicochemical properties, toxicological properties, analytical and bio analytical methods used to evaluate it Also mainly focused on review of process related impurities, enantiomeric separation, metabolites of modafinil in various biofluids and pharmaceutical formulations using HPLC, LC-MS, GC-MS, CE, HPTLC and spectrophotometric methods and challenges that remain in order to ensure the quality.

2.0 Physical-chemical properties

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 $C_{15}H_{15}NO_2S$, 273.35 daltons, respectively. This is slightly soluble or practically insoluble in water, slightly soluble in ethanol and sparingly soluble in methanol. Its melting point is 160-165°C. It is a white to off-white, crystalline powder according to Bio Pharmaceutical classified system. It belongs to a class of drugs known as diphenylmethanes, which are stimulants that provide long-lasting mental arousal [11].

3.0 Pharmacological activities

Modafinil is also recommended for patients suffering from excessive sleepiness, promoting wakefulness, shift work sleep disorder, obstructive sleep apnea/hypopnea syndrome, and potential to treat narcolepsy. It is also like other stimulants, not only increases the release of

monoamines but also enhance hypothalamic histamine levels. It is also used for the treatment of attention-deficit hyperactivity disorder, depression [12], parkinson's disease [13] and disease-related fatigue. It is a memory-improving [14] and mood-brightening psycho stimulant. It is being prescribed to millions of people for "off-label" or "lifestyle" uses [15].

Modafinil is not indicated for complaints of lack of energy or fatigue; but it appears to be very useful for patients. It has been used to treat hypersomnia; a disorder in which patients lack the capacity for meaningful sleep and may require ten or more hours per day. Recent studies have found that modafinil may help recovering cocaine addicts fight their addiction [16, 17]. Modafinil may have low abuse potential despite its stimulant effects. It is an active metabolite of adrafinil. It has proven to be more interesting than adrafinil for inducing wakefulness. It has been shown that (R)- and (S)-forms have the same pharmacological activity as the racemate, but its major metabolite, modafinil acid does not possess any wake promoting activity [18]. This is due to the terminal amide group participates importantly in the biological activity, as observed in the biologically active peptides [19].

4.0 Enantiomeric impurities

Modafinil has sulfoxide group, which exhibits chiral nature, even though, it does not have chiral proton. The special property obtained due to the lone pairs present on the sulfur atom. All organic sulfoxides have a pyramidal space configuration with an isolated pair of electrons occupying the pseudo tetrahedron centre. Due to their high energetic barrier $\sim 40 \text{ Kcal mol}^{-1}$, their conformation is enantiomers.

Enantiomers of a chiral drug have identical physicochemical properties in an achiral environment. In a chiral environment, one enantiomer may exhibit different chemical and

pharmacologic behaviour than the other enantiomer. Since, all living systems are themselves chiral (e.g., carbohydrates, amino acids, and lipids), each of the enantiomers of a chiral drug can behave differently in vivo. Hence, the enantiomers may display distinct pharmacokinetics, toxicities, and metabolic profiles. Therefore, not only knowing the activity of enantiomer but also taking right enantiomer is very important during the medication. In these cases, it is critical to distinguish the single enantiomer from racemic form because they may differ in their dosages, side effects, efficacies and even indicated use. It is well known that in case of a chiral drug administered as a pure enantiomer, its antipode is considered as an impurity [20].

5.0 Pharmacokinetics

In case of modafinil, the enantiomers are both biologically active and it is currently available as racemate under the trade name of provigil®, modavigil®, modiodal®, alertec® and modalert®, respectively. However, R-enantiomer has an apparent steady-state oral clearance 3-fold less than that of S-enantiomer and shows a longer half-life $t_{1/2}$ (10-14h) compared with the S-enantiomer (3-4h) [21, 22]. It has recently resulted in the so-called chiral switch from commercialisation of the racemic compound to marketing the R-enantiomer of modafinil, called armodafinil [23]. It is sold under the trade name of Nuvigil® and Acronite®, respectively. Cephalon, Inc. has announced that armodafinil lasted longer than regular racemic modafinil [24]. Since, the majority of the clinical effects resulting from racemic modafinil administration might, theoretically, be attributable to armodafinil. It is of interest to determine whether the clinical effects observed with racemic modafinil can be achieved with administration of armodafinil alone [25].

6.0 Bioavailability

It is essentially insoluble in water (which poses problems with respect to bioavailability and administration in experimental animals). When modafinil is given orally in a single dose, absorption is slow and T_{max} is between two and four hours [26]. The consumption of food with modafinil does not alter its pharmacokinetic profile [27]. It was found that modafinil metabolizes to modafinil acid, hydroxy modafinil and modafinil sulfone during *in vivo* studies. Only the modafinil acid and modafinil sulfone metabolites have been found in the plasma and urine samples of human subjects. However, both of these metabolites are pharmacologically inactive. Urinary elimination of unconverted modafinil is low (~ 10% of the administered dose). The elimination half-life of modafinil is somewhere between ten and thirteen hours. When multiple doses of modafinil are given orally (200 mg/d), steady state plasma concentration is achieved within two to four days. Enzyme induction is not triggered at this dose level [28].

7.0 Toxicity studies

Modafinil has a low level of toxicity. Its LD₅₀ value is higher than 1 g/kg in rodents (when administered orally) and ~400 mg/kg in canines. Acute and chronic toxicity levels were found to be low with modafinil: when it is administered for 25 weeks to rats in doses of 20 and 50 mg/kg/d, no toxic effects are observed. Similarly, *in vitro* and *in vivo* mutagenesis tests were revealed that no anomalies. The life-long carcinogenicity studies conducted in experimental animals (rats and mice) have produced no suspicious signs of cancer. Gold and Balster et.al., found that modafinil was 200 times less potent than L-ephedrine and D-amphetamine in rhesus monkeys [29].

8.0 Mechanism of Action:

The mechanism involved in the pharmacological control of the narcoleptic symptoms was explained using canine model. These findings could open new therapeutic pathways for human narcolepsy. The pharmacological properties of modafinil are different from than that of the other vigilance-enhancing molecules viz., ephedrine and amphetamine, respectively [30, 31]. After more than three decades of research, scientists are still trying to figure out just how it manipulates the brain [32]. Its mode of action is complex and still uncertain, although studies suggest that it increases wakefulness by activating R-1 noradrenergic transmission or central alpha 1-adrenergic stimulation in relation to the behaviour stimulating effect of modafinil; studies with experimental animals [33].

Modafinil increases dopamine release in the rat nucleus accumbens via the involvement of a local GABAergic mechanism [34]. Studies have proposed that it indirectly modulates the release of γ -aminobutyric acid (GABA) in areas of the brain that regulate sleep and wake cycle in humans and animals [35]. Other researchers suggested that the presynaptic activation of dopamine transmission (DAT) is a key pharmacological event in mediating the wake-promoting effects of currently available CNS stimulants [36]. Several studies suggested that modafinil also modulates the activity of histamine, hypocretin, R-adrenergic and glutamate receptors, respectively [37].

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, orexin

activation and indirect inhibition of noradrenalin reuptake in the ventrolateral preoptic nucleus (VLPO) [38].

9.0 Synthetic Methods of modafinil

Chatterjee et.al., developed a facile synthetic procedure for synthesis of modafinil and its two metabolites [39-41]. Becue et al. evaluated the chemical structure of by products during the synthesis of modafinil by LC-MS but, the method was not validated [42].

10.0 Analytical methods

Table 1 shows analytical and bio analytical methods for determination of modafinil, related impurities and its metabolites reported in the literature. Figure. 1 shows chemical structures of modafinil, armodafinil and its metabolites.

10.1 Calculation of binding constants of modafinil

Capillary Electrophoresis (CE) has been recognized as a powerful technique for enantiomeric separation due to the high separation efficiency, low reagent consumption and rapid analysis times. Enantioseparation is typically achieved by stereo-specific interactions between the chiral selector and analyte. Binding constants and thermodynamic parameters of host-guest complexes are of most important in many areas of research in order to control the complexation equilibrium. That helps in the better understanding of molecular interactions, separation mechanism, in the prediction of migration behaviour and enhancing drug bioavailability [43, 44].

Azzam et.al., developed a method for enantioselective quantification of modafinil in pharmaceutical formulations by capillary electrophoresis [45]. In other publication, the binding

constants were quantified for the enantiomers of modafinil with the negatively charged chiral selector sulfated- β -CD (S- β -CD) using capillary electrophoresis technique [46].

10.2 Quantification of enantiomers

Some methods are available for quantification of enantiomeric purity of modafinil in bulk drugs and pharmaceutical formulation by chromatographic and spectroscopic techniques. This is due to one isomer is biologically active while the other enantiomer is not. As such, development of single enantiomeric drug candidates has become standard practice in the current pharmaceutical industry. Pharmaceutical companies develop chiral active pharmaceutical ingredients (APIs) often require techniques that can quickly assess the enantiomeric purity of a compound throughout the drug development process.

The most of the methods employed in the literature have been chiral HPLC in biological fluids. Drouin et al., discussed optimization of chromatographic conditions viz., the effect of organic modifier and buffer concentration for separation of enantiomers of modafinil on a chiral-AGP column, but, not quantified the enantiomers [47]. Overall, from an industrial pharmaceutical analytical perspective, $^1\text{H-NMR}$ spectroscopic techniques still remain quite useful and convenient when compared with chiral HPLC and CE techniques. Therefore, Rao et.al., evaluated the performance of (R)-(-)- ∞ -Methoxy Phenyl Acetic Acid as a chiral shift reagent for resolution and determination of modafinil enantiomers in bulk drugs and formulations by $^1\text{H NMR}$ Spectroscopy [48].

10.3 Determination of process related impurities

Few methods address to the problem of separation and determination of all process related impurities, which are most likely to be present in the pharmaceutical formulation of

modafinil. Rao et al, developed a reversed phase liquid chromatographic method (RPLC) for separation and quantification of process related substances of modafinil in bulk drugs [49]. In other study, Rao et.al., synthesised and optimized LC-MS/MS conditions for reaction monitoring of modafinil, adrafinil and intermediates, respectively [50].

10.4 Development of stability indicating assay methods

Few stability indicating assay methods were developed and validated in bulk drugs and formulations [51-55]. But, none of them was discussed about structures of degradation products and their degradation pathways. Younus et.al., developed a method for determination of venlafaxine and modafinil in individual tablet formulation using RPLC [56]. Seshamamba et.al., developed a simple spectrophotometric method for determination of modafinil using 1,2-naphthoquinone-4-sulphonate and 2,4-dinitrophenol [57]. Pandya et.al., developed a stability indicating HPTLC method for quantification of modafinil in the bulk drugs and tablet dosage form [58].

11.0 Bio analytical methods

11.1 Quantification of modafinil in biological fluids

Gorman et.al., quantified modafinil and its metabolites such as modafinil acid and modafinil sulfone in human plasma using HPLC utilizing liquid-liquid extraction [59]. Schwertner et.al., developed a RPLC method for quantification of modafinil in plasma and urine [60]. Tseng et.al., developed a method for determination of modafinil in human urine by gas chromatography-mass spectrometry [61]. Bharatiya et.al., developed a LC-MS method for the quantification of modafinil in human plasma [62].

11.2 Quantification of modafinil enantiomers in biofluids

Cass et al., developed an enantioselective HPLC assay method that would be reliable for quantification of modafinil enantiomers and its metabolites viz., (\pm)-modafinil acid and modafinil sulphone by direct human plasma injection through bidimensional achiral–chiral chromatography technique [63]. In other publication, a HPLC method for determination of enantiomers of modafinil in human plasma utilizing solid phase extraction was studied [64]. Gorman et.al., studied quantification of D- and L-enantiomers of modafinil in human plasma utilizing liquid–liquid extraction by HPLC [65]. Donovan et.al., developed a method for chiral analysis of modafinil in human serum and also studied pharmacokinetic profile [66]. Rao et.al., developed a HPLC method for separation and estimation of adrafinil and modafinil enantiomers on Chiralcel OJ-H column in rat serum and urine using solid-phase extraction. The elution order of the enantiomers was studied by a polarimeter connected to PDA detector [67].

11.3 Quantification of modafinil metabolites in biofluids

Modafinil is metabolized into 2-benzylhydrilsulfinylacetic acid (modafinil acid) and its sulfone form (modafinil sulphone) in plasma samples. The two metabolites are biologically inactive. Burnat et.al., quantified modafinil and its two metabolites in human plasma using HPLC through solid phase extraction procedure [68]. McKinney et.al., quantified the modafinil and its major metabolite in equine urine by LC-MS, because it is being thermally labile and unsuitable for gas chromatography/mass spectrometry (GC/MS) analysis [69]. Dubey et.al., studied the screening and confirmation of modafinil, adrafinil, and modafinil acid structures under EI-GC-MS & ESI-LC-MS/MS [70]. Moachon et.al., developed a HPLC method for quantification of modafinil and its acid metabolite (i.e., modafinil acid) in human plasma [71].

12.0 Conclusions

This review describes modafinil properties, its narcolepsy activities, pharmacokinetic studies, and its therapeutic use. It also presents an overview of the analytical and bio analytical methods for quantification of modafinil and its enantiomers not only in bulk drugs but also in pharmaceutical formulations. Pharmaceutical formulations have to obey the law and ensure their efficacy without a raise in risk of the life and treatment of the consumer. Therefore a strict quality control of this drug under study must be done rigorously. So many stability indicating assay methods available, but none of them studied about the characterization of degradation products and their degradation pathways. Analytical techniques viz., HPLC, LC-MS, GC-MS, CE, HPTLC and spectrophotometric methods were used for quality control of modafinil in various matrices.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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Table 1. Analytical and bio analytical methods for determination of modafinil, related impurities and its metabolites reported in the literature

S. No	Column	Mobile phase	Matrices	Range, LOD, LOQ	Method	λ (nm)	Ref
1	Ultrabase CS, (15 cm x 4.6 mm, 5 μ m)	Acetonitrile:0.1 M ammonium acetate(40:60 v/v)	Process related Impurities	NA	HPLC	MS	42
2	Silica capillary (50 μ m x56 cm)	1 M NaOH,	Formulations	Range: 7.5 - 150 μ g/mL LOD:(+) MDL: 1.97 μ g/mL LOQ : (+) MDL 5.97 μ g/mL LOD:(-) MDL: 1.89 μ g/mL LOQ : (-) MDL 5.74 μ g/mL	CE	PDA-225 nm	45

3	Silica capillary 50 mm x 56 cm, (detection length 8.5 cm)	1M NaOH	Enantiomeric inclusion complexes	NA	CE	PDA-225 nm	46
4	NA	Chiral shift reagent:(R)-(+)- α -Methoxy phenyl acetic acid , Solvent: CDCl_3	Bulk Drugs and Formulations	Range: 0.50 to 5.00 mg/mL LOD: 0.02 $\mu\text{g/mL}$ LOQ 0.06 mg/ mL	^1H NMR	499.13 MHz	48
5	Kromasil C_{18} (250 mm \times 4.6 mm, 5 μm)	Acetonitrile: 0.02M ammonium acetate , Gradient elution mode	Bulk drugs	Range: 0.25–2.0 $\mu\text{g/mL}$ LOD: 0.06 ng/mL LOQ: 2.00 ng/mL	RP-HPLC	PDA -225 nm	49
6	Kromasil C_{18} (150 mm \times 4.6 mm, 5 μm)	0.02 M ammonium acetate : Acetonitrile, Gradient elution mode	Bulk drugs	Range: 25–100 ng/mL LOD 6.8 ng/mL LOQ 20.5 ng/mL	RPLC	ESI-MS/MS	50

7	Hypersil ODS C ₁₈ (250 mm x 4.6 mm; 5µm)	1.36g KH ₂ PO ₄ in 550 ml of water and 0.3g K ₂ HPO ₄ 450ml of water: Acetonitrile (55:45 v/v)	Bulk drug and formulation	Range:20-120µg/ mL LOD:2.356 µg/ mL LOQ:7.140 µg/ mL	RP-HPLC	UV- 210 nm	51
8	Phenomenex ODS C ₁₈ (250 mm x 4.6 mm,5µm)	0.2 % acetic acid in methanol and water (90:10, v/v), pH 4.0	Bulk drug and formulation	Range: 10-100µg/ mL LOD: 0.2µg/ mL LOQ 0.8µg/ mL	RP-HPLC	UV- 225 nm	52
9	Thermo Hypersil C ₁₈ (250 mm x 4.6 mm, 5µm)	2.0gm KH ₂ PO ₄ and 1.0 gm/L Octane Sulfonic acid: Acetonitrile (35:65, v/v)	Bulk drug and formulation	Range: 0.2–1.0% of 40 µg/mL LOD: 0.17 µg/ mL LOQ 0.26 µg/ mL	HPLC	UV – 210 nm.	53
10	Hibar C ₁₈ (250 mm x 4.6 mm, 5µ)	Acetonitrile and water (50:50 v/v)	Tablet dosage form.	Range:1–700 µg/ mL LOQ :0.1 µg/ mL	RP-HPLC	UV – 220 nm	54

11	Hypersil ODS C ₁₈ (250 mm x 4.6mm, 5µm).	Acetonitrile and phosphate buffer (45:55, v/v)	Tablet dosage form.	Range: 20 to 120µg/mL	RP-HPLC	UV-220 nm	55
12	Waters symmetry C ₁₈ (250 mm x 4.6 mm, 5µ)	Ammonium acetate (pH : 4.0 :10 % methanol in acetonitrile, (60:40, v/v)	Formulation	Range: 1.0 - 50 µg/ mL	RP-HPLC	PDA- 225 nm	56
13	NA	Reagent: 0.5% 1,2 Naphthoquinone-4-sulphonic acid : 0.1% 2,4 Dinitrophenol	Tablet formulations.	Range: 8-60 µg/mL LOD: 0.258 µg/mL LOQ:0.860 µg/mL	Digital spectrophotometer	UV- 430 nm	57
14	Silica gel 60 F254 (10 cm x10 cm, 50µm)	Ethyl acetate, acetone and methanol (7:2:1 v/v/v)	Bulk drug and formulation	Range: 80-320 ng /spot LOD: 15 ng /spot LOQ:50 ng/spot	HPTLC	Densitometry-232 nm	58

15	Hypersil BDS-phenyl (150 mm × 2 mm, 5µm)	20 mM phosphate buffer (pH 2.5): methanol (64:36, v/v).	Human Plasma	Range: 0.100 to 20.0 mg/mL LOQ: 0.100 mg/mL	HPLC	UV – 235 nm	59
16	Symmetry C ₁₈ (250 mm × 4.6 mm, 5µm)	Methanol:water:acetic acid(500:500:1, v/v/v)	Plasma and urine	Range: 0.1–20.0 µg/mL LOD: 0.1 µg/mL	HPLC	PDA- 220 and 233 nm	60
17	Capillary column,(2.5 m×0.25 mm×0.33 µm)	Helium-carrier gas	Human Urine	Range: 1.25–40 µg/mL LOD: 0.109 µg/mL LOQ 0.363 µg/mL	GC	MS/MS	61
18	Ascentis C ₁₈ (150 mm ×4.6mm, 5µm)	Methanol: 2mM ammonium acetate: glacial acetic acid (35:65:0.1% , v/v/v)	Human plasma	Range: 30.8 to 80 ng/mL LOD: 1 ng/mL LLOQ 30.8 ng/mL	LC	ESI-MS/MS	62

19	APS Nucleosil (150 mm ×4.6 mm ,7µm) with RAM octyl BSA(100 mm ×4.6 mm, 10 µm)	KH ₂ PO ₄ : methanol : acetonitrile, Gradient elution mode	Human plasma	Range: 0.73–2.6% LOD:(+) MDL: 0.050 µg/mL LOQ :(+) MDL:0.080 µg/mL LOD:(-) MDL: 0.100 µg/mL LOQ :(-) MDL: 0.150 µg/mL	Bi- dimensional HPLC	UV- 240 & 228 nm	63
20	APS- Nucleosil (150 mm×4.6 mm,7µm)	Acetonitrile: water (25:75, v/v)	Plasma	Range: 0.15–3.0 µg/mL LOD: 0.005 µg/mL LOQ :0.02 µg/mL	HPLC	UV- 228 nm	64
21	ChiraDex E β- cyclodext rin (250 mm ×4.6mm,5 µm)	20mM phosphate Buffer (pH 3.0): acetonitrile (84:14, v/v)	Human plasma	Range: 0.100 to 15.0mg/ mL LOQ0.100 mg/ mL	HPLC	UV- 225 nm	65

22	β -cyclodextrin column	Gradient elution mode	Human Serum	LOD: 0.01 $\mu\text{g}/\text{mL}$ LOQ (0.5 $\mu\text{g}/\text{mL}$)	HPLC	UV - 225nm.	66
23	Chiralcel OJ-H (250 mm \times 4.6mm, 5 μm)	n-hexane-ethanol (62:38, v/v)	Rat serum and urine	Range: 1.20–500mg/mL LOD:(+) MDL:0.40 $\mu\text{g}/\text{mL}$ LOQ : (+) MDL 0.41 $\mu\text{g}/\text{mL}$ LOD:(-) MDL: 1.20 $\mu\text{g}/\text{mL}$ LOQ : (-) MDL 1.22 $\mu\text{g}/\text{mL}$	NP- HPLC	PDA- 225nm	67
24	Waters Symmetry (150 mm \times 4.6mm, 5 μm)	Acetonitrile :0.05 M phosphate Buffer(pH 2.6)	Human plasma	Range: 0.5–20.0 mg mL LOD: 0.01 mg/ mL LOQ 0.20 mg/ mL	HPLC	UV - 225nm.	68

25	Polaris C ₁₈ (100 mm × 3mm, 2μm)	25mM formate: 5% ethanol, Gradient elution mode	Equine urine	Range: 50–800 ng/mL LOD: 100 ng/mL LOQ: 300 ng/mL	HPLC	MS	69
26	InertcilC ₁₈ (300 mm × 4.6 mm, 5μm)	1% Formic acid and acetonitrile, Gradient elution mode	Human Urine	Range: 100-1000 ng/mL LOD: 250 ng/mL for GC/MS LOD: 100 ng/mL for LC/MS/MS	GC & LC	ESI-MS/MS	70
27	M-Bondapak C ₁₈ (300 mm x 3.9 mm, 10 μm).	Acetonitrile:water:acetic acid (150:420:12, v/v/v).	Human plasma	Range: 0.1-20 mg/mL LOD: 0.04 mg/mL LOQ: 0.13 mg/mL	HPLC	UV- 236 nm	71

NA: Not Available

HPLC= High Performance Liquid Chromatography

MS: Mass spectrometry

ESI: Electro spray Ionization

UV: Ultra Violet Detector

ACCEPTED MANUSCRIPT

GC: Gas Chromatography

LC: Liquid Chromatography

PDA: Photo Diode Array

HPTLC: High Performance Thin Layer Chromatography

RPLC: Reverse Phase Liquid Chromatography

NPLC: Normal phase Liquid Chromatography

CE: Capillary Electrophoresis

NMR: Nuclear Magnetic Resonance Spectroscopy

LOD: Limit of Detection

LOQ: Limit of Quantification

λ (Abs)= wavelength

nm: nano meter

mm: milli meter

μm : micro mete

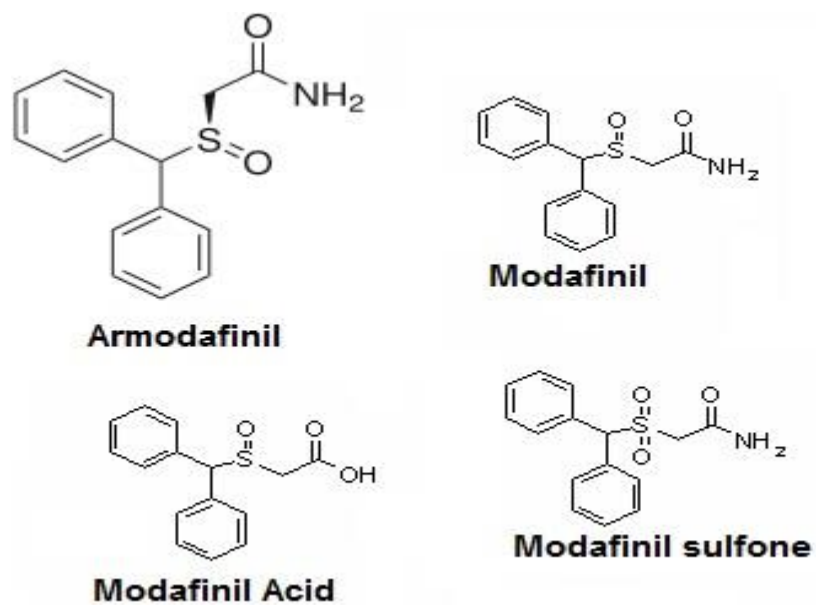


Fig. 1: Chemical Structures of Modafinil, Armodafinil and its metabolites