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Determination of modafinil in plasma and urine by reversed phase high-performance liquid-chromatography

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Abstract

Modafinil (Provigil) is a new wake-promoting drug that is being used for the management of excessive sleepiness in patients with narcolepsy. It has pharmacological properties similar to that of amphetamine, but without some of the side effects associated with amphetamine-like stimulants. Since modafinil has the potential to be abused, accurate drug-screening methods are needed for its analysis. In this study, we 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.
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Keywords: Modafinil; Provigil; High-performance liquid-chromatography; Plasma analysis; Urine analysis

1. Introduction

Modafinil (2-[(diphenylmethyl)sulfinyl] acetamide) is a unique wake-promoting drug that is being used for the management of excessive daytime sleepiness in patients with narcolepsy [1–4]. It is clinically and pharmacologically distinct from other central nervous system stimulants in that it produces long lasting waking effects without behavioral modification, addictive attributes, or sleep rebound [3]. It mimics the effects of amphetamines by producing a very high quality of wakefulness, but without some of the common side effects associated with amphetamine-like stimulants.

Since modafinil is a central nervous system stimulant, it has the potential to be abused. Simple and accurate drug abuse screening methods are needed for analyzing modafinil in urine and plasma. Likewise, therapeutic drug monitoring methods may be needed for analyzing plasma modafinil concentrations especially in elder individuals and in individuals with renal impairment. Age and gender have been shown to effect modafinil clearance and the clearance of modafinil has been shown to be slower in individuals with renal impairment [1]. Both the D- and L-forms of modafinil have been shown to have pharmacological activity, however, the major metabolite, modafinil acid, does not possess any wake-promoting activity [2] (Fig. 1).

Several HPLC methods have been developed for the analysis of modafinil and its metabolites in plasma and urine [5–9]. The methods have been used primarily for

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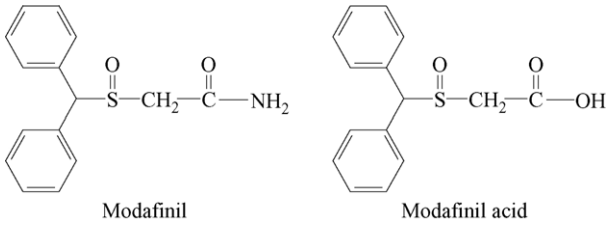

Fig. 1. Chemical structures of modafinil and modafinil acid.

pharmacokinetic studies of modafinil and its enantiomers [5–7]. In those procedures, samples were extracted with hexane–dichloromethane–acetic acid (55:45:2, v/v/v) or by solid phase extraction and analyzed on either phenyl columns or β-cyclodextrin columns. In this study, we describe a relatively simple ethyl acetate and ethyl acetate–acetic acid (100:1, v/v) extraction procedure for analyzing modafinil in urine and plasma and a mobile phase consisting of methanol–water–acetic acid (500:500:1, v/v/v) that is compatible with most reversed phase columns. We also evaluated the diagnostic accuracy of the analytical method by determining modafinil in urine of individuals who had taken 200 or 400 mg of modafinil or a placebo.

2. Experimental

2.1. Chemicals

Provigil tablets were obtained from Cephalon, Inc., (West Chester, PA, USA). (Phenythio)acetic acid, 3-acetamidophenol, and carbamazepine were obtained from Aldrich Chemical Company (Milwaukee, WI, USA), Sigma Chemical Company (St. Louis, MO, USA), and US Pharmacopeia (Rockville, MD, USA), respectively. HPLC grade methanol and ethyl acetate were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Deionized water was prepared with a Barnstead Nanopure II deionizer (Barnstead, Inc., Dubuque, IA, USA). Human blood plasma used in preparing the plasma modafinil calibrators was obtained from our medical center blood bank. The urine samples used in preparing the urine calibrators were obtained from human volunteers.

2.2. Preparation of modafinil standards and urine and plasma calibrators

Modafinil stock standard in methanol (1.0 mg/mL) was prepared by pulverizing 10 Provigil tablets (Cephalon, Inc.) each containing 100 mg of modafinil. One hundred milligrams of the pulverized modafinil powder were then extracted twice with 50 mL of methanol on an Eberbach shaker for 30 min. The extracts were centrifuged at 3000 rpm for 20 min, made to volume with methanol, and stored at $4 \pm 3\,^{\circ}C$.

Negative plasma and urine pools were used for preparing the modafinil calibrators. Prior to preparing the calibrators, the plasma and urine pools were extracted and analyzed by HPLC to insure that they did not contain co-extractable substances that might interfere with the analysis of modafinil or the internal standard. Plasma and urine modafinil calibrators (0.1, 1.0, 5.0, 10.0, 20.0 μg/mL) were prepared by adding appropriate volumes of the modafinil stock standard to separate16 mm × 125 mm centrifuge tubes. After evaporation of the methanol, several 10 mL aliquots of a negative plasma or urine pool were added to resuspend the modafinil. The aliquots were mixed and then transferred to a 100 mL volumetric flask. The calibrators were made to volume with the negative plasma or urine and stored at $4 \pm 3\,^{\circ}C$.

2.3. Subjects

Sixteen subjects took 200 mg of modafinil, 400 mg of modafinil or a placebo on different weeks. Two-hour post-dose urine samples were then obtained from the individuals taking the two doses of modafinil and from the sham controls. The urine samples were stored at $-80 \pm 4\,^{\circ}C$ prior to analysis. Plasma samples from 28 individuals who had taken 325 mg of aspirin and 1.0–4.0-h post-dose urine samples from 20 individuals who had taken 975 mg of acetaminophen were also analyzed to determine if aspirin or acetaminophen interfered with the assay. The Institutional Review Board approved the study protocol and written informed consent was obtained from all individuals participating in the study.

2.4. Extraction procedure

Five milliliters of urine modafinil calibrators, negative urine control, and urine test samples or 2 mL of the plasma modafinil calibrators, negative plasma control, and plasma test samples were pipetted into separate 16 mm × 150 mm or 16 mm × 125 mm glass screw-capped centrifuge tubes. Twenty micrograms of (phenylthio)acetic acid internal standard (1.0 mg/mL in methanol) was added to each 2 mL plasma sample and 50 μg of (phenylthio)acetic acid was added to each 5 mL urine sample (note: for the analysis of urine samples, the (phenylthio)acetic acid internal standard was added after extraction of the urine samples with ethyl acetate). The samples were vortex mixed for about 10 s. Ten milliliters of ethyl acetate was added to the urine samples and 10 mL of ethyl acetate–acetic acid (100:1, v/v) was added to the plasma samples. The samples were shaken on an Eberbach shaker for about 30 min on slow speed and then centrifuged at 3000 rpm (rotor #216, CentraGP8R) for 20 min. The plasma and urine extracts were transferred with a Pasteur pipet to conical centrifuge tubes and evaporated under nitrogen at $60 \pm 4\,^{\circ}C$ in a Zymark TurboVap evaporator (Caliper Life Sciences, Hopkinton, MA, USA). After drying, the samples were reconstituted in 700 μL of the HPLC mobile phase; vortex mixed for about 10 s, and centrifuged at 3000 rpm for 5.0 min. The extracts were then transferred to HPLC injection vials.

2.5. Instrumentation

Modafinil was analyzed on Waters HPLC system consisting of a 996 Photodiode Array Detector, 600E Controller, 717 Autosampler, and Millennium 2010 Chromatography Manager (Waters, Milford, MA, USA). Analysis was performed on a 4.6 mm × 250 mm Symmetry C18 reverse column, (Cat. No. WATO 54215) with methanol–water–acetic acid (500:500:1, v/v) as the mobile phase. The following HPLC parameters were used for the analysis of modafinil: injection volume, 10–30 μL; column flow rate, 1.0 mL/min; spectra recording, 220 and 233 nm.

3. Results and discussion

3.1. Evaluation of internal standards

For the analysis of modafinil, we evaluated 3-acetamidophenol, (phenylthio)acetic acid, and carbamazepine as possible internal standards (Table 1). 3-Acetamidophenol and carbamazepine could be readily extracted from urine and plasma with ethyl acetate; however, (phenylthio)acetic acid required extraction under acidic conditions or it had to be added to the extraction solvent after extraction. 3-Acetamidophenol had a retention time shorter than that of modafinil whereas (phenylthio)acetic acid and carbamazepine had a retention time longer than that of modafinil (Table 1). (Phenylthio)acetic acid was selected as the internal standard for the analysis of modafinil because co-extractable material was not found to interfere with its analysis (Figs. 2 and 3).

Table 1
Retention times of modafinil and candidate internal standards[a]

Internal standard	Retention time (min)
3-Acetamidophenol	3.6
Modafinil	11.5
3-(Phenylthio)acetic acid	15.2
Carbamazepine	15.7

[a] Mobile phase: methanol–water–acetic acid (500:500:1, v/v); column flow rate: 1.0 mL/min.

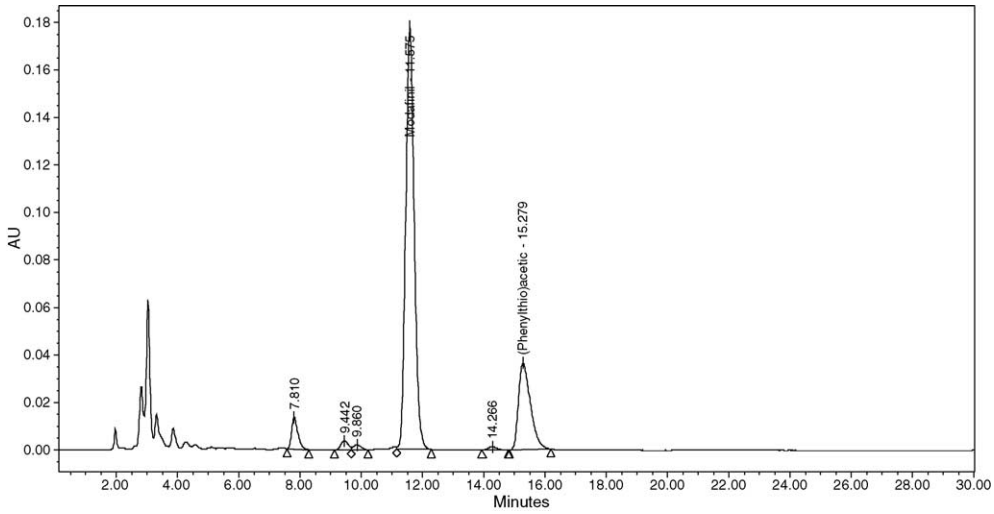

Fig. 2. HPLC chromatogram of modafinil (19.8 μg/mL) and the internal standard, (phenylthio)acetic acid, extracted from urine.

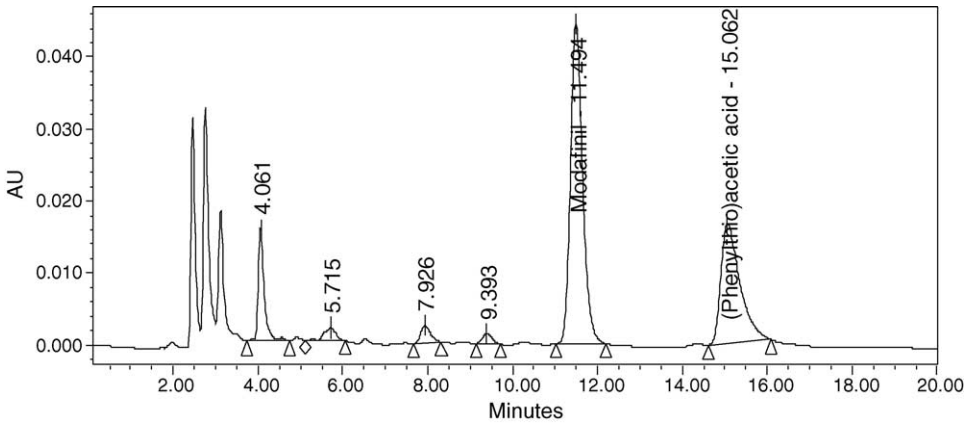

Fig. 3. HPLC chromatogram of modafinil (10.0 μg/mL) and the internal standard, (phenylthio)acetic acid, extracted from plasma.

3.2. Evaluation of extraction solvents

Ethyl acetate was used as the extraction solvent for the analysis of modafinil in urine. We later used ethyl acetate–acetic acid (100:1, v/v) as an extraction solvent for the analysis of modafinil in plasma samples. Even though ethyl acetate extracted modafinil with recoveries of about 80%, we chose ethyl acetate–acetic acid (100:1, v/v) because it could extract (phenylthio)acetic acid when added to plasma as an internal standard. One slight disadvantage of using ethyl acetate–acetic acid (100:1, v/v) over ethyl acetate is that the ethyl acetate–acetic acid extracts were slightly more turbid than the ethyl acetate extracts when they were reconstituted in the mobile phase. The turbidity could be removed by centrifuging the reconstituted samples at $3000 \times g$ for 5 min before transferring to the injection vials. We also selected ethyl acetate–acetic acid (100:1, v/v) as the extraction solvent because it would likely be able to extract modafinil acid. Modafinil acid can potentially be used to confirm the intake of modafinil in drug abuse cases. It was not analyzed in this study because it was not commercially available.

3.3. Method validation

The recoveries of modafinil from urine and plasma were $80 \pm 3\%$ and $98.9 \pm 2\%$, respectively (Table 2). The recoveries of modafinil from urine and plasma were determined by comparing the concentrations of the extracted urine and plasma calibrators to those of the modafinil standards. The limit of quantitation was 0.1 μg/mL when modafinil was measured at 220 or at 233 nm and for both urine and plasma samples. The within-day CV's for urine and plasma samples containing 10.0 μg/mL modafinil were 2.5 and 2.4%, respectively and the between-day CV's were 5.4 and 3.5% for the urine and plasma samples, respectively. Similar recoveries and CV's were found at modafinil concentrations of 1.0 and 5.0 μg/mL. Modafinil standard curves using urine or plasma calibrators were linear from 0.1–20.0 μg/mL at both 220 and 233 nm ($r^2 \geq 0.999$). The analytical sensitivity for modafinil was about 1.7 times higher at 220 nm than at 233 nm, however, there were slightly more interferences from co-extractables at 220 nm than at 233 nm. Therefore, 233 nm was selected as the wavelength for the analysis of modafinil. The 2-h post-dose urine modafinil concentrations were almost identical when analyzed at 220 and 233 nm (13.5 ± 9.2 μg/mL at 220 nm and 13.6 ± 9.6 μg/mL at 233 nm; $r^2 = 0.999$; $n = 32$).

Table 2
Analytical parameters of the HPLC method for analyzing plasma and urine modafinil[a]

Sample	Urine	Plasma
Internal standard	(Phenylthio)acetic acid	(Phenylthio)acetic acid
Recovery[a]	$80.0 \pm 3\%$	$98.9 \pm 2\%$
Detection limit	0.1 μg/mL	0.1 μg/mL
Within-day CV[a]	2.5%	2.4%
Between-day CV[a]	5.4%	3.5%
Linearity	0.1–20.0 μg/mL	0.1–20.0 μg/mL

[a] Based on extraction of 5 mL of urine or 2 mL of plasma containing 10.0 μg/mL of modafinil and analyses at 233 nm.

3.4. Application of the method

The diagnostic accuracy of the HPLC method for detecting modafinil in urine was determined by analyzing urine samples from 32 individuals who had taken modafinil and in urine from 16 individuals who had taken a placebo. The samples were analyzed without knowledge of drug administration. All 16-placebo urine samples were correctly classified as being negative for modafinil at a modafinil concentration <0.1 μg/mL. Except for one sample, all 32 2-h post-dose urine samples from individuals taking either 200 or 400 mg of modafinil were found to contain modafinil. This urine sample was reanalyzed by the same procedure and found to be negative presumably due to lack of compliance. Therefore, all 48 of these urine samples were correctly classified. Serum from individuals taking 325 mg of aspirin or urine samples from individuals taking 975 mg of acetaminophen tested negative for modafinil. In addition, no interfering substances (≥0.1 μg/mL) were found in plasma samples from 28 randomly selected individuals when analyzed at 233 nm.

The analytical procedure for the analysis of modafinil in plasma and urine is simple, reproducible, and accurate. The analytical method differs from the previously published procedures in that those procedures were developed mainly for pharmacokinetic studies of modafinil and its enantiomers [5–7]. The analytical method reported here also differs from the previously published methods in that we used ethyl acetate or ethyl acetate–acetic acid (100:1, v/v) as the extraction solvent whereas the previous procedures used hexane–dichloromethane–acetic acid, 55:45:2, v/v/v [4] or a solid phase absorbent [6,8]. We also used methanol–water–acetic acid (500:500:1, v/v/v) as the mobile phase whereas the previous procedures used acetonitrile–0.02 M potassium phosphate buffer (30:70, v/v; pH 2.5 for plasma samples and pH 4.0 urine samples) [5–7], a combination of acetonitrile and orthophosphoric acid [8] or acetonitrile and acetic acid [9]. The analytical method reported here, eliminates the possible toxic hazards associated with the use of dichloromethane and acetonitrile and the effects of acidic phosphate salt buffers on the chromatographic columns and pumps. In addition, the within-day and between-day CV's of this method were lower than the 15% achieved with the previous method [5]. The sensitivity of the present method was found to be comparable to those reported previously [6,8], however, the sensitivity can be increased further by resuspending the extract in a smaller volume of mobile phase, e.g., 100 μL, by injecting a larger volume, or by using a smaller internal diameter column.

4. Conclusions

The procedure described here is diagnostically accurate and correctly classified the presence or absence of modafinil in 48 blinded urine samples. The lower limit of detection of the method is 0.1 μg/mL. We have found no problems with interferences in urine samples of 32 individuals taking either 200 or 400 mg of modafinil or in 16 placebo urine samples. In addition, we did not find any interfering substances in plasma samples from 28 individuals taking aspirin or in 1.0–4.0-h post-dose urine samples from individuals who had taken 975 mg of acetaminophen. The HPLC method can be used for screening plasma and urine samples for the presence of the modafinil and it can be used for pharmacokinetic studies and for therapeutic monitoring. In addition, it can be readily adapted to the liquid-chromatographic–mass-spectrometric (LC–MS) analysis of modafinil.

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