Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Pharmaceutical and Biomedical Analysis 39 (2005) 1042–1045
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Detection of modafinil in human urine by gas chromatography–mass spectrometry

Ying Lung Tseng [a,*], Victor Uralets [b], Chien-Tzong Lin [a], Fan-Hsin Kuo [a]

[a] *Institute of Pharmacology and Toxicology and Doping Control Center, Tzu Chi University, 701 Section 3, Chung Yan Road, Hualien, Taiwan*
[b] *Quest Diagonistics, Las Vegas, NV, USA*

Received 14 March 2005; received in revised form 15 April 2005; accepted 20 April 2005
Available online 29 June 2005

Abstract

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.

Keywords: Modafinil; Doping control; Stimulants; GC–MS

1. Introduction

Modafinil (*d,l*-2-[(diphenylmethyl)sulfinyl]acetamide, $C_{15}H_{15}NO_2S$, MW 273) is a new drug developed by Cephalic Inc. (West Chester, PA, USA) and was approved by the FDA in the US under the trade name Provigil® (Fig. 1) [1]. This relatively new drug possesses stimulating and awaking properties. It has been used for treating excessive daytime sleepiness or narcolepsy without interfering with nocturnal sleep [2,3]. The exact mechanism of modafinil's action in the brain is not yet fully understood. Studies have proposed that modafinil indirectly modulates the release of gamma aminobutyric acid (GABA) in areas of the brain that regulate sleep and wake cycle in both humans and animals. Additionally, it does not appear to have central and peripheral side effects associated with conventional dopaminergic psychostimulants [4–7].

The first doping violation involving modafinil was reported in 2003 at the World Track and Field Championship [8]. Later in 2004, modafinil was added to the stimulant-drug list prohibited by the World Anti-Doping Agency (WADA) [9]. Previous detection of this prohibited drug was performed analytically by HPLC [1,2,10–13]. HPLC is commonly used in sport-related testing as an initial screen for certain drugs (e.g. diuretics), because it requires less sample preparation [14]. Unfortunately, data obtained from HPLC may not provide sufficient specificity to inarguably identify the drug in question. Therefore, utilizing the outstanding accuracy of the GC–MS technique provides unequivocal identification of banned substances. To the best of our knowledge, GC–MS analysis of modafinil has not yet been reported in the literature. We describe a simple procedure for GC–MS detection and quantification of modafinil and profiling of its excretion pattern in human urine.

* Corresponding author. Tel.: +886 3 856 4640; fax: +886 3 857 8167.
E-mail address: ying@mail.tcu.edu.tw (Y.L. Tseng).

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.04.050

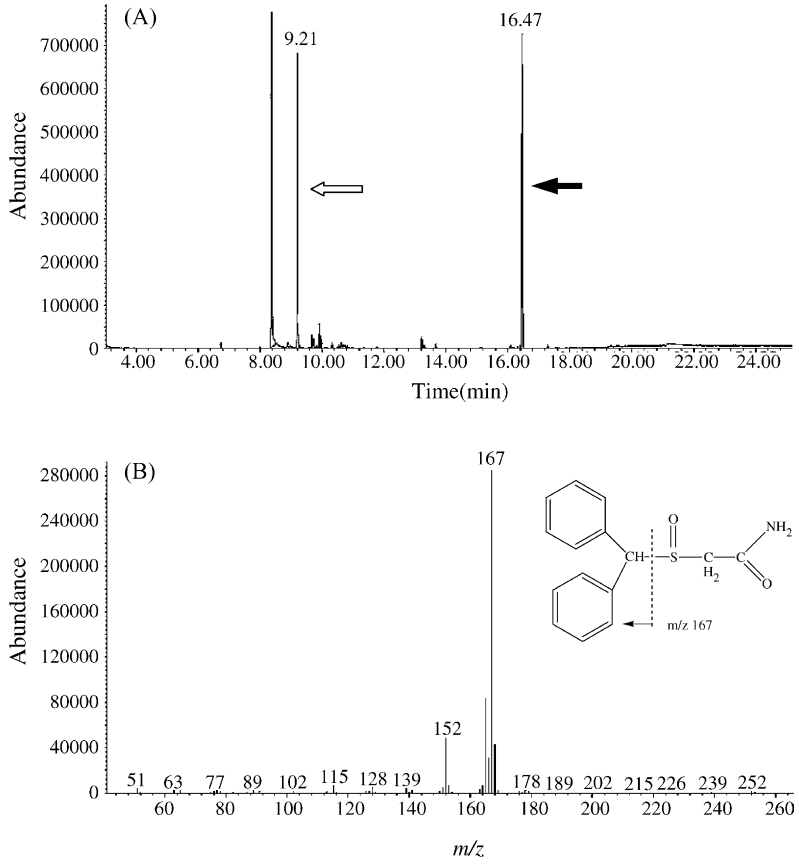

Fig. 1. GC–MS analysis of modafinil reference standard spiked in urine. Total ion chromatogram (A) and EI mass spectrum of authentic modafinil following liquid–liquid extraction from urine (B). Filled arrow and open arrow denotes modafinil and internal standard, respectively.

2. Materials and methods

2.1. Chemicals and standards

All reagents were of analytical grade. Ethyl acetate, potassium carbonate, sodium hydrogen carbonate, and phenazine (as an internal standard, IS) were purchased from Mallinckrodt (St. Louis, MO, USA). *t*-Butyl-methyl ether (TBME) was purchased from Riedel-de Haën (Seelze, Germany). 3,3-Diphenylpropylamine was obtained from Aldrich (Milwaukee, WI, USA). Modafinil was purchased from Sigma (St. Louis, MO, USA). Provigil® tablets were manufactured by Cephalon Inc. (West Chester, PA, USA).

2.2. Instrumentation and conditions

A Hewlett-Packard HP 5890 GC interfaced with a 5972 mass selective detector (MSD) was used in this study. A capillary column (HP-5MS cross-linked 5% diphenyl and 95% dimethylpolysiloxane 25 m × 0.25 mm × 0.33 μm film thickness) was used for separation. Helium was used as a carrier gas with split flow rate of 1.1 mL/min. The GC–MS injection port and the interface temperatures were set at 250 and 300 °C, respectively. The initial temperature was 90 °C followed by raising 15 °C/min to 240 °C and 10 °C/min to 300 °C (holding time 5 min). The analysis was carried out in a full scan mode with electron impact ionization at 70 eV and mass spectra were obtained by scanning from *m/z* 50 to 550. One microliter of sample was injected with the autosampler.

2.3. Drug administration and urine collection

The human subject research review committee approved this study. Three healthy adult male volunteers took part in the excretion study. Each volunteer was orally administered a clinical dose (100 mg) of Provigil® (modafinil) tablet urine specimens were collected at 0, 2, 4, 6, 8, 12, 24, 48, 72, 96 and 120 h post administration. All the urinary samples were stored at −20 °C before analysis.

2.4. Sample preparation

To serve as an authentic standard, modafinil was dissolved in methanol (1 mg/mL) and kept at −20 °C until used. For the urinary samples, 1 mL aliquot, 50 μL phenazine (IS), 0.6 g $NaHCO_3$:K_2CO_3 (3:2 w/w, pH 9–9.5) and 1 mL TBME:2-propanol (9:1 v/v) were added to a 15 mL glass tube, followed by shaking and centrifugation at 2200 rpm for 9 min. The

organic layer was transferred and evaporated to dryness under a slow flow of nitrogen gas. The sample extract was reconstituted with 100 μL ethyl acetate before GC–MS analysis.

2.5. Quantification of modafinil in urine

The modafinil calibration solutions were spiked in triplicates with appropriate amounts of authentic reference standards to the drug-free urine. One set of standards, including 1.25, 2.5, 5, 10, 20, 40 μg/mL, was prepared for constructing a modafinil calibration curve. The calibration curve for linear regression analysis of analyte was constructed by plotting the peak area ratio of the modafinil reference standard and the internal standard versus the concentrations of the analyte.

3. Results

3.1. Analysis of modafinil reference standard

Modafinil standard solution was prepared by dissolving 1 mg of modafinil reference standard in 1 mL methanol. To characterize the nature of modafinil in GC–MS analysis, drug-free urine spiked with modafinil standard (50 μg/mL) was pretreated according to the procedure described in Section 2. The results revealed that one chromatographic peak appeared at retention time (RT) of 16.50 min with a relative retention time (RRT) of 1.79. The mass spectrum of modafinil was characterized by ions of *m*/*z* 167 (base ion), 165 and 152 (Fig. 2).

3.2. Limit of detection, limit of quantification and recovery

The limit of detection (LOD) was defined as a signal-to-noise ratio of approximately 3–1. The LOD of modafinil was determined to be 0.109 μg/mL when analyzed with a single diagnostic ion (*m*/*z* 167) by GC–MS. The limit of quantification (LOQ) of the assay was 0.363 μg/mL as determined mathematically by the concentration of modafinil produced a S/N ratio of 10 using a single diagnostic ion (*m*/*z* 167). To determine extraction efficiency (or recovery) of the sample

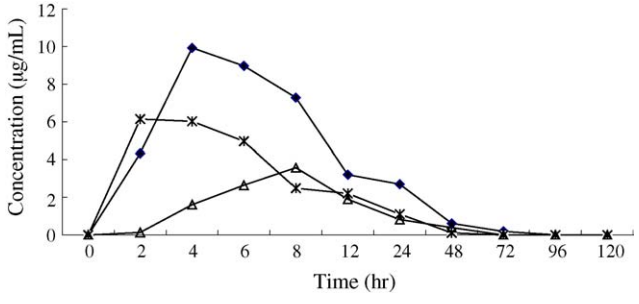

Fig. 2. Urinary excretion profiles of modafinil after oral administration to three volunteers.

Table 1
Recovery of modafinil in human urine

Concentration (μg/mL)	Recovery (% ± S.D.)	CV (%)
2.5	65.5 ± 5.0	7.64
5	74.8 ± 2.8	3.81
20	72.0 ± 6.3	8.70

Table 2
Accuracy and precision for the analysis of modafinil

Concentration (μg/mL)	Intra-assay		Inter-assay	
	Target (%)	CV (%)	Target (%)	CV (%)
2.5	96	10.7	97.3	6.8
5	86	5.1	93.3	3.7
20	82	2.3	88.0	3.7

preparation procedure using TBME liquid–liquid extraction, three target concentrations of modafinil with three replicates for each concentration were analyzed. The recovery at each analyte concentration was determined by comparing the peak area of the extracted analyte to that of unextracted analyte. The recovery of the modafinil target concentrations at 2.5, 5 and 20 μg/mL were 65.5% (7.6% CV), 74.8% (3.8% CV) and 72.0% (8.70% CV), respectively (Table 1).

3.3. Linearity, accuracy and precision

Three batches of 1 mL aliquots were used for linearity assay as described in Section 2. The assays were linear over the range of 1.25–40 μg/mL with the correlation coefficient (R^2) of 0.9995, 0.9983 and 0.9993, respectively. The accuracy was determined by the deviation between detected and added analyte concentration; the precision was determined by the coefficient of variation (CV) of six replicates at the same concentrations of the analyte. The measurements of accuracy and precision of modafinil are shown in Table 2. The intra-assay accuracy and precision of modafinil at target concentrations of 2.5, 5 and 20 μg/mL within a single analytical batch were 96, 86 and 82% with precision (CV%) of 10.7, 5.1 and 2.3%, respectively. The interassay accuracy and precision were determined from three separate analytical runs, using the same concentrations as were used in the intra-assay studies. The interassay accuracy measured for modafinil concentrations at 2.5, 5 and 20 μg/mL were 97.3, 93.3 and 88.0% with precision (CV) of 6.8, 3.7 and 3.7%, respectively.

3.4. Analysis of urinary samples from excretion study

In the excretion study, three adult male volunteers were orally administered a clinical dose (100 mg) of modafinil and their urine samples were collected at different time points. Prior to GC–MS analysis urine samples were prepared as that described in Section 2. The results indicated that one peak in the total ion chromatograms (TIC) appeared at RT 16.46 min (RRT, 1.79) in both modafinil standard control and modafinil administered urine samples. The EI mass spectra from these

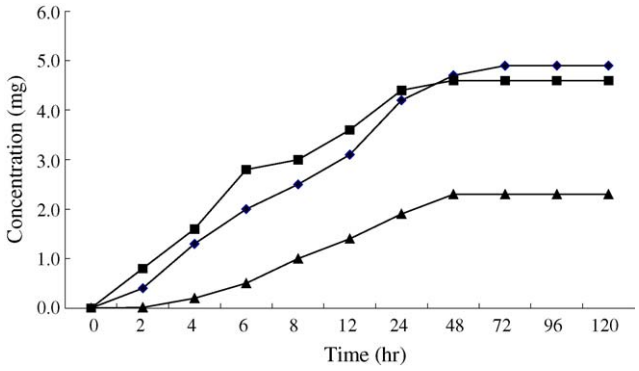

Fig. 3. Cumulative excretion profile of modafinil in urine from the three volunteers.

two samples showed identical characteristic ions, *m*/*z* 167 (base ion), 165 and 152 (data not shown).

3.5. Excretion profile and cumulative amount of modafinil in urine following oral dosing

The times to reach peak concentration in the urine samples of the three volunteers varied at 2 h (6.14 μg/mL), 4 h (9.93 μg/mL) and 8 h (3.58 μg/mL) (Fig. 2). To obtain cumulative amount of unchanged modafinil in urine from each volunteer, amount of the analyte at each time point was first obtained by multiplying the analyte concentration (in μg/mL) in urine by total volume of urine (in mL) voided during that time period. The cumulative amount of unchanged modafinil in urine was then determined by the sum of the amount of modafinil derived from each time point. The cumulative amounts of modafinil in urine obtained from the three volunteers were 4.9, 4.6 and 2.3 mg, respectively (Fig. 3).

4. Discussion

The extraction of modafinil from drug-spiked urine samples and urine samples collected from the excretion study were carried out in the absence of hydrolysis and derivatization steps. Under these conditions one single peak, identified as unchanged modafinil, was present in the GC chromatogram identified on the basis of three characteristic ions at *m*/*z* 167 (base ion), 165 and 152 were shown in the mass spectrum. This procedure was simple to carry out and yielded clear and consistent results as opposed to the procedures involving acid or enzyme (β-glucuronidase from *E. coli*.) hydrolysis and derivatization (data not shown). In these unreported studies, we found that in both control and urine samples, acid hydrolyzed and trifluoroacetic anhydride (TFAA) derivatized sample preparation gave multiple GC chromatographic peaks (more than eight different peaks). This finding also occurred in β-glucuronidase hydrolyzed and *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA)/trimethyliodosilane (TMIS) derivatized urine samples. Consequently, the multiple chromatographic peaks caused a great difficulty in data interpretation.

Our GC–MS results differed from previous studies using HPLC in that we identified only one chromatographic peak rather than two (modafinil acid and modafinil sulfone) [2,13,15]. The reasons for this discrepancy may be attributed a number reasons and merits further investigation.

The results of excretion studies from the three volunteers showed inter-individual differences in excretion profiles with the modafinil concentration peaking at 2 h (6.14 μg/mL), 4 h (9.93 μg/mL) and 8 h (3.58 μg/mL). The cumulative amounts of modafinil in urine from the three volunteers were 4.9, 4.6 and 2.3 mg. These results suggested that approximately 2–5% of unchanged parent drug out of a 100 mg dose of modafinil (one Provigil® tablet) administered to the volunteers was excreted into urine as early as 2 h. The drug was cleared within approximately 48–72 h for all the volunteers tested. These results were in agreement with one report that less than 10% unchanged drug was present in urine following a single oral dose of modafinil [15].

Acknowledgements

The authors acknowledge the invaluable comments provided by Drs. Tony J.F. Lee and R.H. Liu in the preparation of this manuscript.

References

[1] S.H. Gorman, J. Chromatogr. B 730 (1999) 1–7.
[2] P. Burnat, F. Robles, B. Do, J. Chromatogr. B 706 (1998) 295–304.
[3] D.B. Biovin, J. Montplaisir, D. Petit, C. Lambert, S. Lubin, Clin. Neuropharmacol. 16 (1993) 46–53.
[4] K. Fuxe, F.A. Rambert, L. Ferraro, W. O'Connor, P.H. Laurent, L.F. Agnati, S. Tanganelli, Drugs Today 32 (1996) 313–326.
[5] D.P.S. Lagarde, G. Anton, Eur. J. Pharmacol. 183 (1990) 1476.
[6] J. Duteil, F.A. Rambert, J. Pessonier, J.-F. Hermant, R. Gombert, Eur. J. Pharmacol. 180 (1990) 49–58.
[7] H. Bastuji, M. Jouvet, Prog. Neuropsychopharmacol. Biol. Psychiat. 12 (1988) 695–700.
[8] S. Hart, Drug shame for US sprint star, Sport.telegraph, August 31, 2003. http://www.telegraph.co.uk/sport/main.jhtml?xml=/sport/2003/08/31/sodrug31.xml.
[9] The World Anti-Doping Code, The 2005 Prohibited List International Standard, World Anti-Doping Agency, September 23, 2004.http://www.wada-ama.org/rtecontent/document/list_2005.pdf.
[10] Y.N. Wong, L. Wang, L. Hartman, et al., J. Clin. Pharmacol. 38 (1998) 971–978.
[11] Q.B. Cass, C.K. Kohn, S.A. Calafatti, H.Y. Aboul-Enein, J. Pharm. Biomed. Anal. 26 (2001) 123–130.
[12] P. Robertson Jr., E.T. Hellriegel, Clin. Pharmacokinet. 42 (2003) 123–137.
[13] G. Moachon, D. Matinier, J. Chromatogr. B 654 (1994) 91–96.
[14] C.N. Yoon, T.H. Lee, J. Park, J. Anal. Toxicol. 14 (1990) 84–90.
[15] Y.N. Wong, S.P. King, D.W. Laughton, G.C. McCormick, P.E. Grebow, J. Clin. Pharmacol. 38 (1998) 276–282.