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Simultaneous determination of modafinil and its acid metabolite by high-performance liquid chromatography in human plasma

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Abstract

A 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 min. 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. To our knowledge this is the first described method for determination of modafinil and its acid metabolite in plasma.

1. Introduction

Modafinil is a new drug developed in France for use in the treatment of narcolepsy. The drug is characterized by an atypical psychopharmacological profile involving central postsynaptic alpha-1 adrenergic receptor modulation (activation) [1]. Modafinil is a 2-benzhydrylsulfinylacetamide. Modafinil is metabolized into 2-benzhydrylsulfinylacetic acid (I), the acid form of modafinil. The acid metabolite is inactive in pharmacological tests. To our knowledge, no report on the development of an analytical method for the quantitation of modafinil and its acid metabolite is available. The present paper describes the first high-performance liquid chromatographic (HPLC) method allowing sensitive and selective analysis of modafinil and its metabolite in plasma at therapeutically relevant concentrations.

2. Experimental

2.1. Materials

Modafinil (I) and the internal standard [bis-(4-fluoro-phenyl)-methylsulfinyl]acetic acid (I.S.), the structures of which are given in Fig. 1, were synthetised in the Research Laboratory of Laboratoire L. Lafon (Maisons-Alfort, France).

Acetic acid and hydrochloric acid were of

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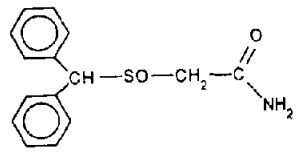
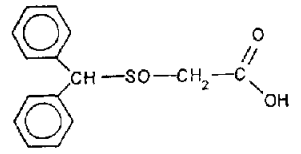
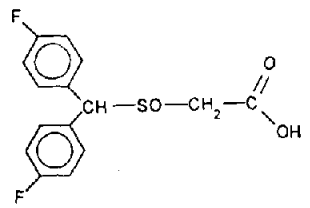


MODAFINIL



I



IS

Fig. 1. Structures of modafinil, I and internal standard (I.S.).

analytical grade. Methanol, acetonitrile and diethyl ether were of HPLC grade. These products were obtained from Merck (Nogent-sur-Marne, France). Purified water (Milli-Q system, Millipore, Saint-Quentin-en-Yvelines, France) was used for all aqueous solutions. Control (blank) human plasma (collected on Li heparinate) was obtained from healthy volunteers free of drugs.

2.2. Standards

Stock standard solutions were prepared by dissolving separately 10 mg of modafinil, 10 mg of I and 10 mg of I.S. in 10 ml of methanol. The working standard solution contained both modafinil and I. The concentrations of each compound were 200, 150, 100, 50, 20, 10, 5, 2 and 1 mg/l. Working solutions were prepared with appropriate dilutions in methanol and stored at 0–4°C during not more than two weeks.

2.3. Instrumentation

The HPLC system consisted of a S400 isocratic pump (Kratos Analytical, Applied Biosystem, Tremblay-les-Gonesses, France), a WISP 710B autosampler (Waters Millipore, Saint-Quentin-en-Yvelines, France), an SM 3200 visible detector (LDC Analytical, Finnigan Mat, Orsay, France) coupled to an ENICA 10 integrator (Delsi Nermag, Argenteuil, France) in the peak-area mode at an attenuation of 8.

The separation was achieved at room temperature on a μBondapak C_{18} column (particle size 10 μm, 300 mm $\times$ 3.9 mm I.D., Waters Millipore). Initial column pressure was 90 bar. The samples were eluted at a flow-rate of 1.4 ml/min with a mixture of acetonitrile–water–acetic acid (150:420:12, v/v/v). The mobile phase was filtered through a Sartorius filter (0.47 μm) prior to use.

The compounds were quantified at a wavelength of 236 nm, using a detector range of 0.005 AUFS.

2.4. Extraction procedure

To 1.0 ml of plasma in a 16-ml glass test tube were added 1 ml of 0.1 *M* HCl, 100 μl of working standard solution, 100 μl of I.S. (4 μg) and 10 ml of diethyl ether. The mixture was shaken for 10 min. The two phases were separated by centrifugation at 4000 *g* for 10 min at 0–4°C. The organic layer was transferred into a clean tube and evaporated to dryness at ambient temperature under a stream of nitrogen. The dry residue was dissolved in 200 μl of eluent, vortex-mixed for 30 s and a 25-μl aliquot was injected onto the HPLC system.

2.5. Calibration

Calibration curves were constructed by adding 20, 15, 10, 5, 2, 1, 0.5, 0.2 and 0.1 μg of modafinil and I together and 4 μg of I.S., to aliquots of control plasma (1 ml) and by ex-

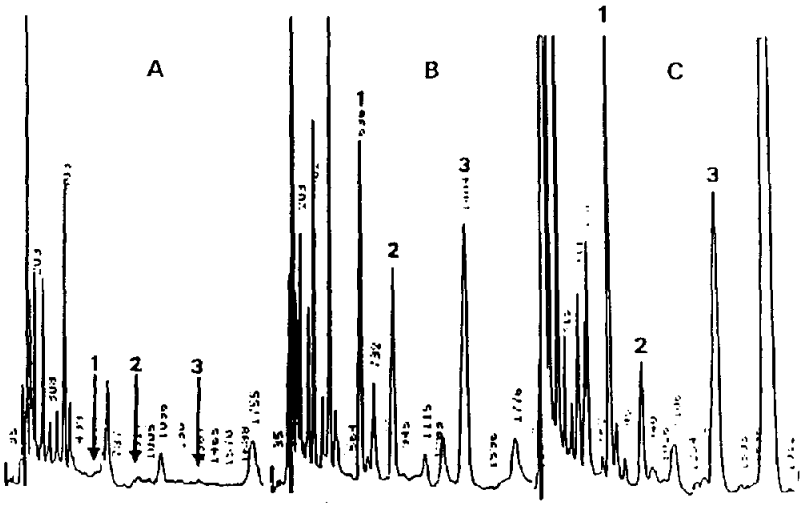


Fig. 2. Chromatograms of extracts. (A) control human plasma; (B) control human plasma spiked with 2 mg/l modafinil, 2 mg/l I and 4 mg/l I.S.; (C) plasma from a patient receiving a 200-mg oral dose of modafinil. Peak 1 corresponds to modafinil, peak 2 to I, and peak 3 to I.S.

tracting these samples. Unweighted least-squares regression lines were generated using peak-area ratios of the compounds to the internal standard against the plasma concentration of the compound. Additionally, spiked plasma samples were processed within each run as a means of quality control.

3. Results and discussion

3.1. Selectivity

Fig. 2 shows typical chromatograms obtained from extracts of blank human plasma (A), plasma spiked with modafinil, I and I.S. (B), and plasma from a patient receiving a 200-mg oral dose of modafinil (C).

Under the conditions described above, the peaks, representing modafinil, I, and I.S., were symmetrical, baseline resolved and separated from the solvent front. Using this method we did not detect any interference with the modafinil, I and I.S. peaks measured in the plasma samples from more than 90 subjects. The retention times of modafinil, I and I.S. were 11.0, 15.0 and 24.0 min respectively. The time run was set at 45 min in order to elute endogenous substances before the next run.

3.2. Linearity

The linearity of the response for each compound was established by analysis of three standard curves over the concentration range 0.1–20 mg/l. Standard curves contained nine points. A statistical test of linearity was performed for each curve separately using an unweighted analysis of variance (ANOVA). The calibration curves for the two compounds were linear over the investigated concentration range, when peak-area ratios (compound/I.S., y-axis) were plotted against concentrations (x-axis), and applied to a least-square regression equation. Analysis of all calibration series showed an excellent linearity at the 0.05 significance level, a correlation coefficient ≥ 0.9978 close to 1 and a y-intercept not significantly different from zero. For modafinil and I, the mean correlation coefficients were 0.9989 and 0.9980 respectively; the standard deviations (S.D.) were 0.0001 and 0.0003; the equations of the mean regression line were

$y = (0.274 \pm 0.003)x + (0.032 \pm 0.016)$ and $y = (0.270 \pm 0.002)x + (0.012 \pm 0.012)$.

3.3. Limits of detection and quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) were determined according to the method of Carr and Wahlich [2]. The LOD was defined as the concentration equal to three times the value of the signal-to-noise ratio. Baseline noise was measured after extraction of ten different blank plasmas. The LOD of the assay was evaluated as 0.04 mg/l for the two compounds. The LOQ was defined as the concentration equal to ten times the value of signal-to-noise ratio. The LOQ of the assay was evaluated as 0.13 mg/l for modafinil and 0.14 mg/l for acid metabolite.

3.4. Recovery

The absolute recovery of each compound was assessed at three concentrations by comparing the peak areas after extraction with the mean peak area obtained from direct injection of the working solutions used to spike the samples. The three concentration were 0.5, 2, and 15 mg/l. Table 1 lists the results of the recovery studies.

The recovery of the I.S. was calculated with 30 peak-area determinations obtained after extraction of the ten plasma samples of each concentration *versus* 30 area determinations by direct injections. I.S. recovery in plasma was $69.3 \pm 7.12\%$ ($n = 30$, mean ± S.D.).

3.5. Intra- and inter-day precisions and accuracy

The intra-day precision ($n = 10$) for 0.5, 2 and 15 mg/l modafinil concentrations was 9.4, 6.8 and 7.3% respectively. Values calculated for I were 10.1, 4.8 and 5.2%, respectively.

The inter-day precision and accuracy were studied using the data of three quality controls analysed over twelve days. The results are shown in Table 2. Precisions expressed as coefficients of variation (C.V.) ranged from 6 to 10.4% and the accuracy defined as (amount found/amount added) × 100 (%) reached approximately 100% for each compound throughout the three concentrations examined.

3.6. Stability

Using the extraction procedure described, we noticed no transformation of modafinil into I *in vitro*. To test the stability of the compounds, spiked plasma samples were either stored at −20°C during 30 days or left on the bench at room temperature for 24 h before extraction or after extraction. Results of these studies were compared with the results of spiked samples which were immediately analysed. Statistical analysis of the differences between concentrations were evaluated by ANOVA. The data in Table 3 show that modafinil and I were stable over a 24-h period at room temperature in extracts; by contrast modafinil and I were not stable over a 24-h period at room temperature in the biological matrix. Modafinil was partially transformed into I. On the other hand, the

Table 1
Recovery of modafinil and I from human plasma

Compound	Concentration (mg/l)	Recovery (mean S.D., $n = 10$) (%)	C.V. (%)
Modafinil	0.5	55.2 ± 7.15	12.95
	2	69.8 ± 6.78	9.71
	15	68.6 ± 7.91	11.53
I	0.5	44.4 ± 7.07	15.92
	2	61.6 ± 6.90	11.20
	15	59.5 ± 8.10	13.61

Table 2
Inter-day precision and accuracy results for plasma samples spiked with modafinil and I

Compound	Concentration (mean ± S.D.) (mg/l)		Precision[a] (%)	Accuracy[b] (%)
	Added	Found		
Modafinil	0.5	0.508 ± 0.053	10.4	102
	2	1.839 ± 0.167	9.1	92
	15	13.259 ± 1.013	7.6	88
I	0.5	0.493 ± 0.044	8.9	99
	2	1.932 ± 0.132	6.8	97
	15	15.330 ± 0.920	6.0	102

[a]Coefficient of variation.
[b](Found/added) × 100.

Table 3
Stability of modafinil and I

Sample	Modafinil (mg/l)	I (mg/l)
Samples immediately analysed	1.99 ± 0.10	2.12 ± 0.001
Samples at room temperature for 24 h before analysis	1.73 ± 0.06[a]	2.32 ± 0.12[a]
Extracts at room temperature for 24 h before analysis	1.91 ± 0.01	2.13 ± 0.01
Samples stored at − 20°C for 30 days	2.05 ± 0.05	2.23 ± 0.03

[a]$p < 0.05$.

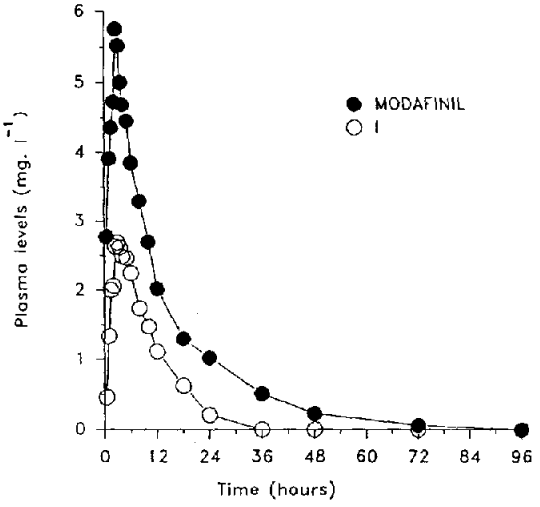


Fig. 3. Plasma concentration–time curve for modafinil and its metabolite, I, after a 200-mg oral dose of modafinil.

stability of both compounds was good after one month storage at −20°C.

3.7. Clinical study

The analytical method was applied to a clinical study with modafinil. Fig. 3 shows a representative plasma concentration–time curve of modafinil and I after oral administration of a therapeutic dose of modafinil (200 mg) to a healthy volunteer. Plasma concentrations of modafinil and I could be measured over a 72-h period allowing good determination of pharmacokinetic parameters.

4. Conclusions

This paper describes an isocratic reversed-phase HPLC method developed for simultaneous

quantification of modafinil and its acid metabolite in plasma. The described analytical method is simple and sensitive. It has a limit of quantitation of 0.13 mg/l for modafinil and 0.14 mg/l for the acid metabolite. The assay is precise and accurate, and gives a linear response in the range 0.1–20 mg/l. The method has been used for the analysis of samples for clinical pharmacokinetic trials in the development of this new drug.

5. References

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