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# High-performance liquid chromatographic determination of modafinil and its two metabolites in human plasma using solid-phase extraction

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**Abstract**

A simple procedure for the simultaneous determination of modafinil, its acid and sulfone metabolites in plasma is described. The assay involved an extraction of the drug, metabolites and internal standard from plasma with a solid-phase extraction using $C_{18}$ cartridges. These compounds were eluted by methanol. The extract was evaporated to dryness at 40°C under a gentle stream of nitrogen. The residue was redissolved in 250 μl of mobile-phase and a 30 μl aliquot was injected via an automatic sampler into the liquid chromatograph and eluted with the mobile-phase (26%, v/v acetonitrile in 0.05 *M* orthophosphoric acid buffer adjusted to pH 2.6) at a flow-rate of 1.1 ml/min on a $C_8$ Symmetry cartridge column (5 μm, 150 mm×3.9 mm, Waters) at 25°C. The eluate was detected at 225 nm. Intra-day coefficients of variation ranged from 1.0 to 2.9% and inter-day coefficients from 0.9 to 6.1%. The limits of detection and quantitation of the assay were 0.01 μg/ml and 0.10 μg/ml respectively. 

*Keywords:* Solid-phase extraction; Modafinil

## 1. Introduction

Modafinil, 2-[(diphenylmethyl)sulphinyl]-acetamide, is a new drug with stimulant and awaking properties. It activates central postsynaptic alpha-1 adrenergic receptors [1]. Modafinil has proven to be effective in the treatment of narcolepsy and hypersomnia without interfering with nocturnal sleep [2,3]. Furthermore, because of its stimulant properties, this drug can be employed as a stimulant doping agent [4,5].

Modafinil is metabolized into 2-benzylhydrylsulfinylacetic acid and the sulfone form of modafinil. The two metabolites are inactive and the sulfone form is in minority in plasma. Another high-performance liquid chromatography (HPLC) method involving liquid–liquid extraction and one of the metabolites has been published [6].

In this work, we describe a simple and sensitive HPLC method to measure modafinil and its two metabolites in human plasma applicable to toxicological and pharmacological studies. The method employs solid-phase extraction (SPE) as sample preparation.

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## 2. Experimental

### 2.1. Analytical standards

Modafinil (Fig. 1), modafinil acid, modafinil sulfone and internal standard were of pharmaceutical purity and obtained from Laboratory L. Lafon, Maison-Alfort (France).

#### 2.1.1. Reagents

All reagents were of HPLC grade and included acetonitrile and methanol (Prolabo, Fontenay s/s Bois, France). Orthophosphoric acid and hydrochloric acid were of analytical grade (Prolabo). Deionized water (Aguettan, Lyon, France) was used for all aqueous solutions.

Control (blank) human plasma (collected on lithium heparinate) was obtained from healthy volunteers free of drugs.

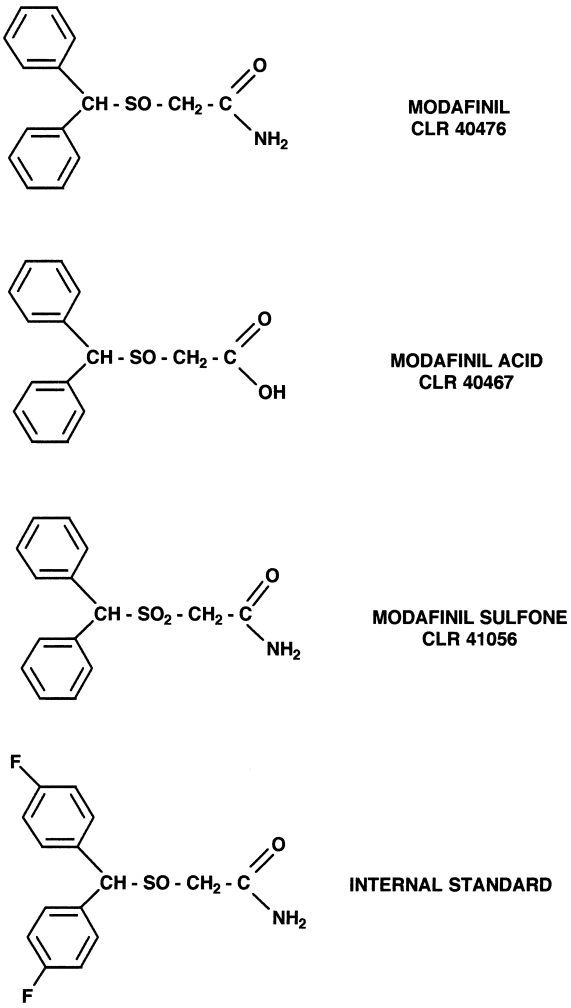


Fig. 1. Chemical structures for modafinil, modafinil acid, modafinil sulfone and internal standard.

#### 2.1.2. Apparatus and chromatographic conditions

The chromatographic system consisted of a Waters 600 reciprocating piston pump, a Waters 717 intelligent sample processor (WISP TM) and a 996 programmable photodiode-array detector (Waters, Milford, MA, USA).

Analysis was performed on a $C_8$ reversed-phase cartridge column (Symmetry cartridge, 5 μm, 150 mm×3.9 mm I.D., Waters) linked to a $C_8$ precolumn (5 μm, 30 mm×3.9 mm I.D., Waters). The separation was carried out at stable temperature (25°C) in an oven.

The isocratic mobile phase used was a mixture of 26% (v/v) acetonitrile in 0.05 *M* orthophosphoric acid solution adjusted to pH 2.6 with sodium hydroxide (10 *M*). This was degassed by vacuum and sonicated before use, and the flow-rate was 1.1 ml/min.

The detector was set at 225 nm. The WISP autoinjector was programmed to run for 40 min per sample, using 10% methanol in distilled water as the rinse solvent, at ambient temperature (25±1°C). Other apparatus used included extraction columns (Varian $C_{18}$, Bond Elut LRC®, Harbor City, CA, USA).

#### 2.1.3. Preparation of standard solutions and calibration samples

Stock solutions of modafinil and its two metabolites were prepared by dissolving accurately weighed quantities in methanol to give a concentration of 1.0 mg/ml. These stock solutions were used to spike the drug-free plasma (obtained from a blood bank) to cover various calibration ranges: 0.5–20.0 μg/ml.

Fresh calibration external standards and extraction processed standards of the plasma standards were run together with 20 unknown samples as a batch processed by the automatic sampler, which was normally operated overnight. Four standards were placed at the beginning and at the end of the batch of

unknowns. This was used to check the linearity and the reproducibility of the chromatographic system.

### 2.2. Extraction method

SPE columns were preconditioned by passing through 2 ml of methanol followed by 2 ml of hydrochloric acid 0.05 *M*. Plasma (1 ml) was pipetted accurately into a 10 ml centrifuge tube and 100 μl of internal standard were added. After the addition of 1 ml of 0.05 *M* hydrochloric acid solution, the whole content was loaded on a SPE column and drawn through by means of the vacuum manifold.

The solid-phase was washed with 2 ml of a mixture of 20% (v/v) ethanol in hydrochloric acid solution 0.05 *M*. Then, the SPE columns were slowly eluted by methanol 3×1 ml. The eluent was evaporated to dryness at 40°C under a gentle stream of nitrogen. The residue was dissolved in 250 μl of mobile phase and an aliquot (30 μl) was injected into the HPLC system via the automatic sampler.

### 2.3. Quantitation

The calibration graphs were constructed by spiking modafinil and its metabolites into drug-free plasma samples. Diluted stock methanolic solutions were prepared to cover the concentration range 0.5–20.0 μg/ml. Between-day standards of concentrations 1 and 10 μg/ml were determined to obtain the between-batch variation of the assays.

The calibration graphs were repeated 6 times for measurement of reproducibility.

### 2.4. Recovery

Various amounts (1, 5, 20 μg) of modafinil and its metabolites were dissolved in 1 ml human blank plasma by adding 100 μl aliquots of appropriate working solutions. These samples were extracted as described but without adding the internal standard. The extraction residues obtained were dissolved in 250 μl of mobile phase containing 5 μg of internal standard and chromatographed.

A second series of samples was prepared simultaneously by extraction of 1 ml aliquots of human blank plasma. Modafinil, its metabolites and internal standard were then added to the extraction residue at the concentration noted above.

The analytical recovery was calculated by comparing the peak-area ratios of modafinil, modafinil acid, modafinil sulfone/internal standard in extracted samples, to the ratios obtained from the samples to which, studied compounds and internal standard had been added after extraction.

## 3. Results and discussion

### 3.1. Performance of the HPLC system

The analytical peaks of modafinil, modafinil acid, modafinil sulfone and internal standard were well resolved (Fig. 2) and the mean±S.D. ($n$=6) of retention times were 10.3±0.4, 12.8±0.3, 25.2±0.5 and 29.2±0.5 min, respectively. No endogenous peaks from blank plasma samples were observed which might interfere with the peak-areas measurement of the analytical peaks in the plasma samples from more than 20 subjects. The run time was set at 40 min in order to elute endogenous substances before the next run.

### 3.2. Recovery

The data presented in Table 1 demonstrate that the three components were extracted quantitatively from human plasma in the concentration range investigated.

In previous study [6], the method based on a liquid–liquid extraction using diethyl ether as the extraction solvent showed recovery from 44.4 to 69.8%. SPE as described the present study seems to improve the mean (range) recoveries of modafinil as is shown in Table 1.

### 3.3. Limits of detection and quantitation

The limit of detection of the assay was 0.01 μg modafinil/ml, 0.02 μg modafinil acid/ml and 0.02 μg modafinil sulfone/ml plasma using 1 ml specimens. For this minimum detectable concentration, a signal-to-noise ratio of approximately 3:1 was observed and concentration calculated with 6 samples.

The limit of quantitation of the assay was evalu-

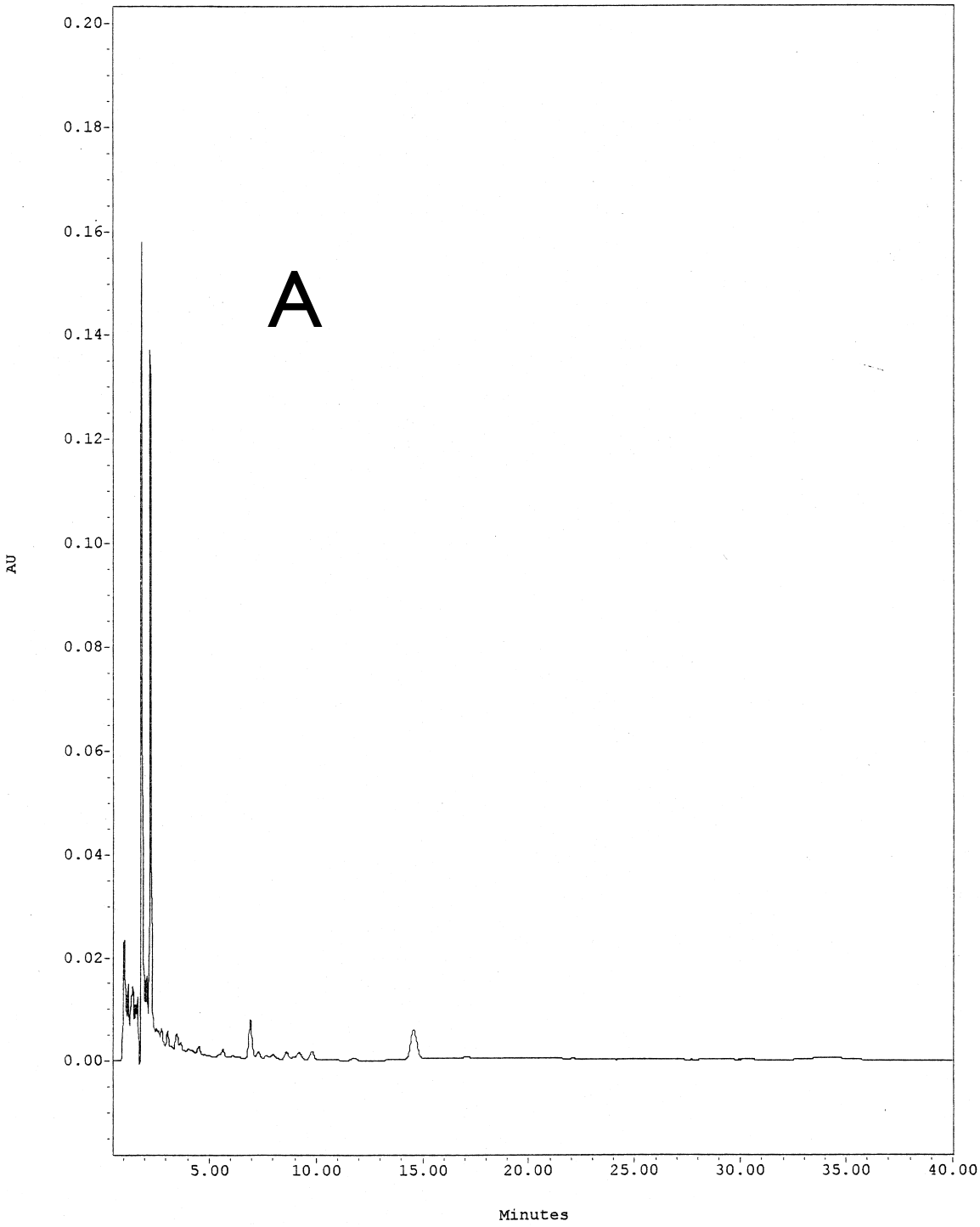


Fig. 2. Chromatograms of the analysis of: (A) drug-free plasma; (B) a standard containig 2.5 μg/ml modafinil, modafinil acid and modafinil sulfone and 10 μg/ml internal standard in plasma; (C) a plasma sample containing 2.47 μg/ml modafinil, 2.30 μg/ml modafinil acid and 0.18 μg/ml modafinil sulfone after administration of a modafinil tablet (200 mg) to a healthy male subject; (D) expanded view of peak 3 from chromatogram C. Peaks: 1=modafinil; 2=modafinil acid; 3=modafinil sulfone; 4=internal standard.

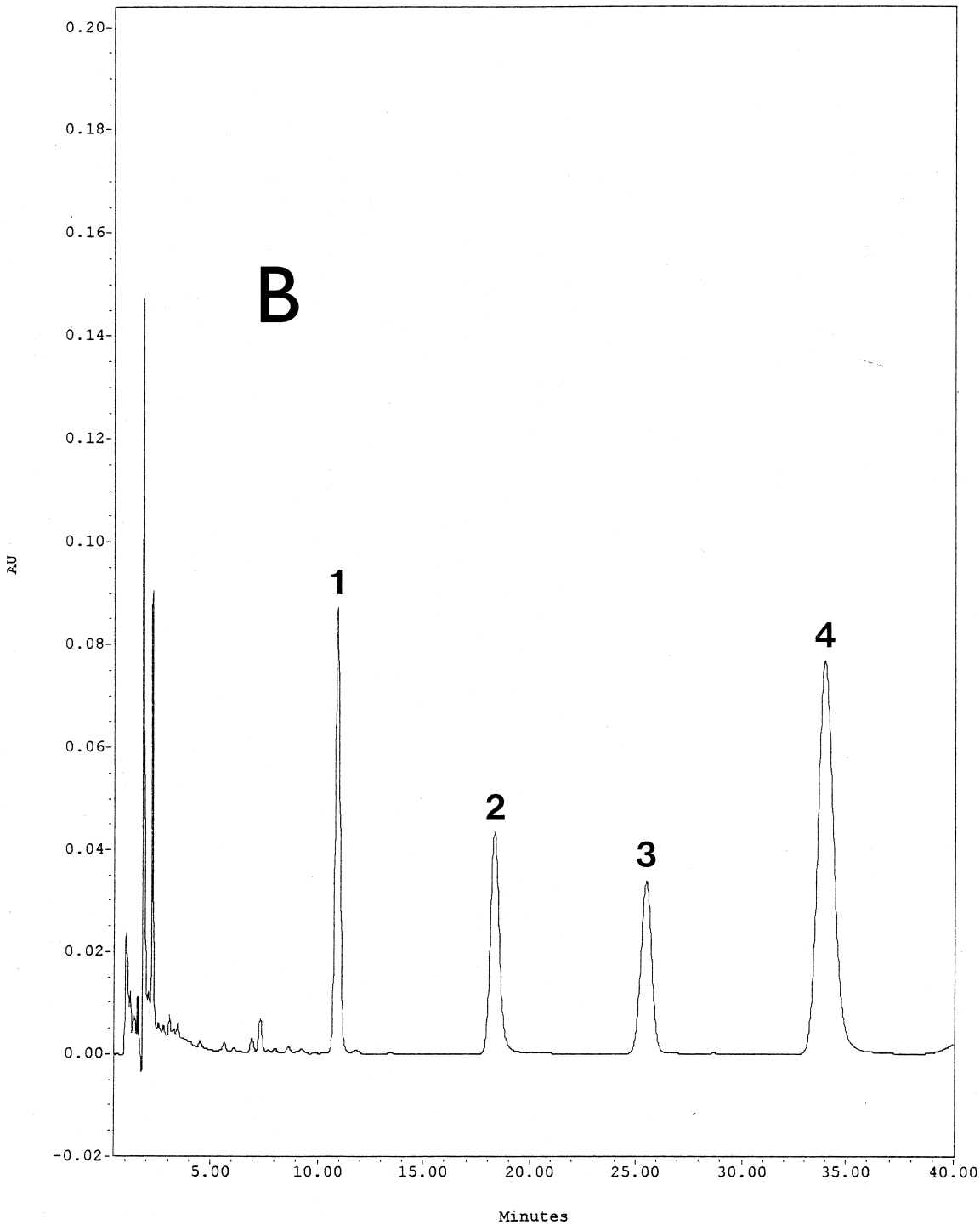


Fig. 2. (*continued*)

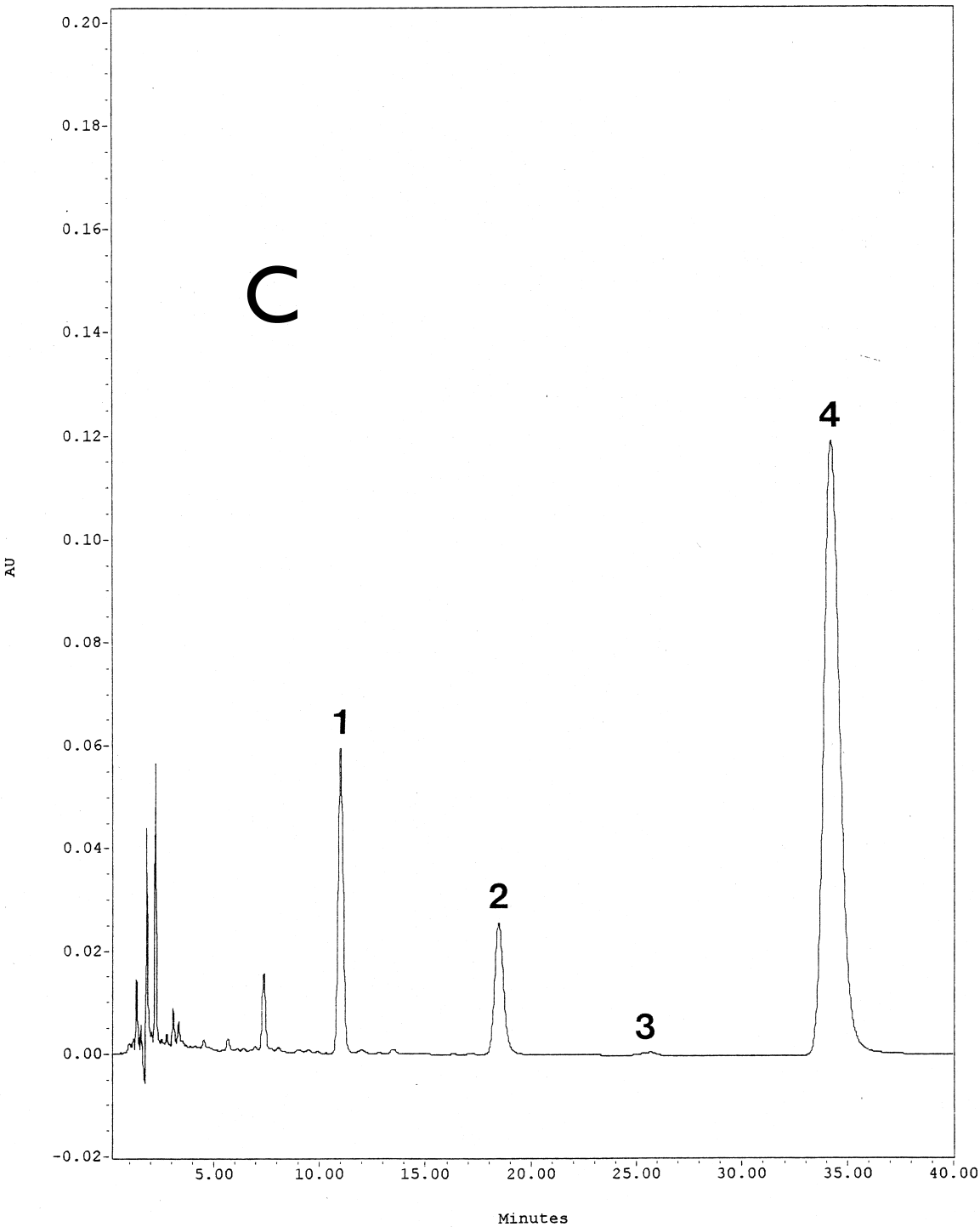


Fig. 2. (*continued*)

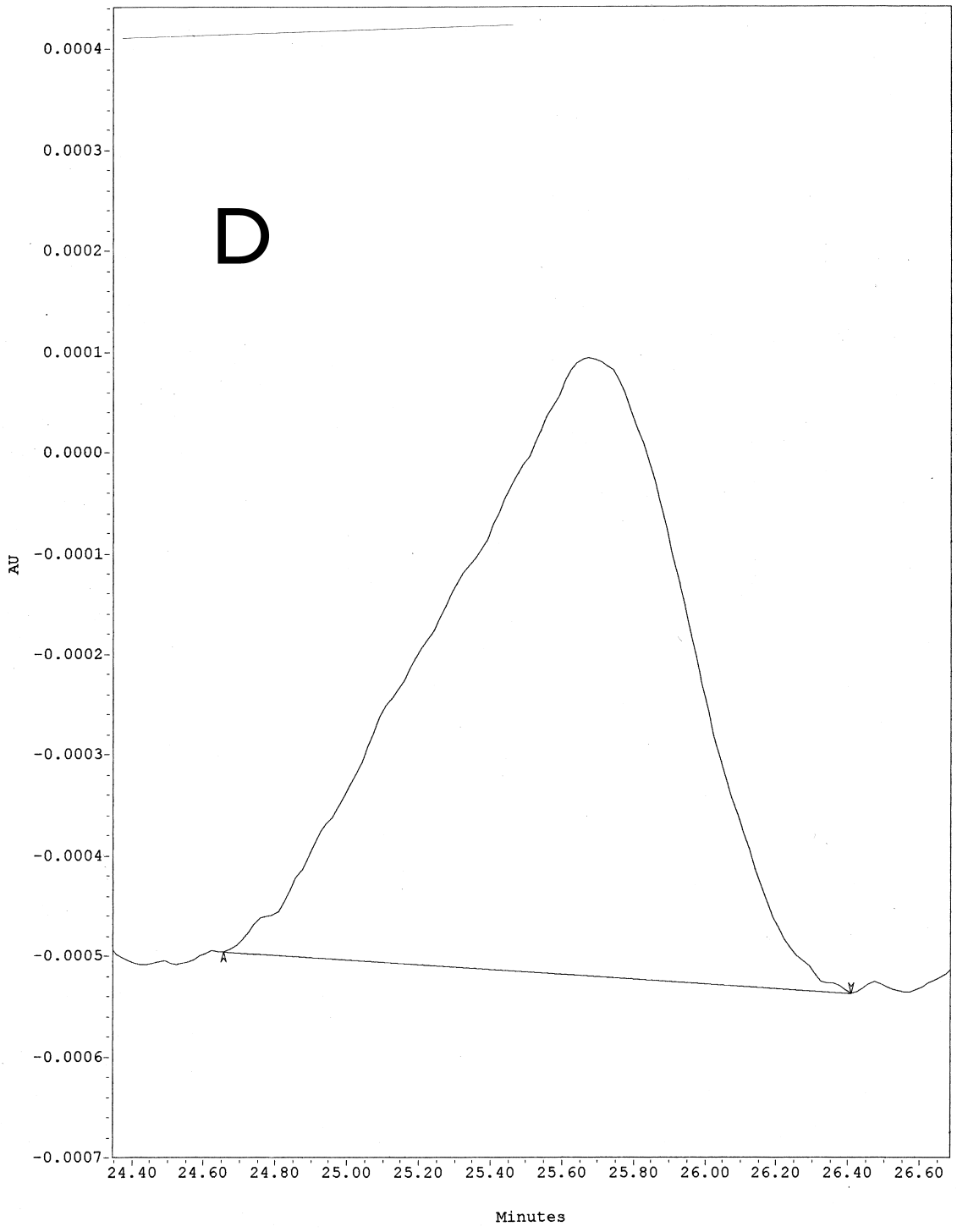


Fig. 2. (*continued*)

Table 1
Recovery of modafinil and metabolites in human plasma ($n=6$)

| Concentrations (μg/ml) | Recovery (%) |
|---|---|
| Modafinil | |
| 1.0 | 89±4 |
| 5.0 | 84±5 |
| 20.0 | 84±3 |
| Modafinil acid | |
| 1.0 | 90±3 |
| 5.0 | 85±4 |
| 20.0 | 84±3 |
| Modafinil sulfon | |
| 1.0 | 87±3 |
| 5.0 | 85±3 |
| 20.0 | 84±5 |

ated as the concentration equal to 10-times the value of signal-to-noise ratio. It was evaluated as 0.10 μg/ml for modafinil, 0.12 μg/ml for modafinil acid and 0.11 μg/ml for modafinil sulfone. However, the practical limit of quantitation, defined here as the minimum concentration that can be measured routinely with acceptable precision and accuracy, was 0.20 μg/ml for modafinil (C.V.=9.8%, $n=6$), 0.25 μg/l for modafinil acid (C.V.=10.3%; $n=6$) and for modafinil sulfone (C.V.=12.8%; $n=6$).

### 3.4. Linearity

Linearity was studied in the range 0.5–20.0 μg/ml plasma corresponding to the ranges observed in clinical samples. Calibration curves were obtained by weighted least-squares linear regression analysis (weight factor $1/y^2$) of the peak-area ratio of modafinil and the metabolites/internal standard versus the concentrations of modafinil and metabolites respectively. A statistical test of linearity was performed for each curve separately using an unweighted analysis of variance. Analysis of all calibration series showed an excellent linearity at 0.01 significant level. The mean correlation coefficients were acceptable (Table 2). The equations of the mean regression line are shown in Table 2.

Table 2
Calibration and precision of the HPLC assay using plasma samples ($n=6$)

| Spiked concentration (μg/ml) | Modafinil | | | Modafinil acid | | | Modafinil sulfone | | |
|---|---|---|---|---|---|---|---|---|---|
| | Measured concentration (mean±S.D.) | C.V.[a] (%) | Accuracy | Measured concentration (mean±S.D.) | C.V.[a] (%) | Accuracy | Measured concentration (mean±S.D.) | C.V.[a] (%) | Accuracy |
| 0.5 | 0.59±0.04 | 6.11 | +0.09 | 0.58±0.03 | 5.51 | +0.08 | 0.48±0.01 | 2.92 | −0.02 |
| 1.0 | 1.19±0.02 | 1.29 | +0.19 | 1.16±0.04 | 3.71 | +0.16 | 1.08±0.04 | 4.05 | +0.08 |
| 2.5 | 2.20±0.02 | 1.59 | +0.06 | 2.49±0.07 | 2.69 | −0.01 | 2.30±0.06 | 2.58 | −0.2 |
| 5.0 | 4.67±0.20 | 4.00 | −0.33 | 4.74±0.12 | 2.65 | −0.26 | 4.88±0.25 | 5.11 | −0.12 |
| 10.0 | 9.83±0.18 | 1.84 | +0.17 | 9.93±0.12 | 1.20 | −0.07 | 10.45±0.20 | 1.94 | +0.45 |
| 20.0 | 20.19±0.28 | 1.37 | +0.19 | 20.13±0.18 | 0.88 | +0.13 | 19.79±0.21 | 1.05 | −0.21 |
| Batch standards ($n=10$) | | | | | | | | | |
| 1.0 | 1.20±0.01 | 1.0 | +0.20 | 1.18±0.01 | 1.25 | +0.18 | 1.07±0.03 | 2.93 | +0.07 |
| 10.0 | 9.96+0.12 | 1.2 | −0.04 | 10.02±0.11 | 1.09 | +0.02 | 10.06±0.19 | 1.89 | +0.06 |
| Calibration graphs | $y=0.0615x+0.038$ | | | $y=0.0594x+0.016$ | | | $y=0.0622x+0.0116$ | | |
| Correlation coefficients ($r$) | 0.9989 | | | 0.9996 | | | 0.9991 | | |

[a] Coefficients of variation.

### 3.5. Precision

The precision (expressed by the coefficient of variation of replicate analyses) and the accuracy (expressed by the deviation between found and added concentration) of the method were evaluated over a concentration range of 0.5–20.0 μg modafinil and metabolites/ml plasma.

The intra-assay reproducibility was determined by analysing 10 specimens of spiked plasma on the same day. The inter-assay reproducibility was obtained by analysing one specimen of a spiked plasma sample on various days, using a separate calibration each day. The data shown in Table 2 demonstrate the good precision of the method over the concentration range investigated. Even the concentration 0.5 μg/ml near the limit of quantitation was measured with sufficient precision when replicate determinations were performed on different days.

### 3.6. Clinical study

The analytical method was applied to a clinical study with modafinil. Fig. 2C shows a representative chromatogram from this study. Fig. 3 shows plasma concentration–time profiles of modafinil, modafinil acid and modafinil sulfone after oral administration of a therapeutic dose of modafinil (200 mg) in a healthy volunteer. The excellent precision of the method allows the accurate determination of pharmacokinetic parameters from plasma concentration–time data. In our study, the pharmacokinetic parame-

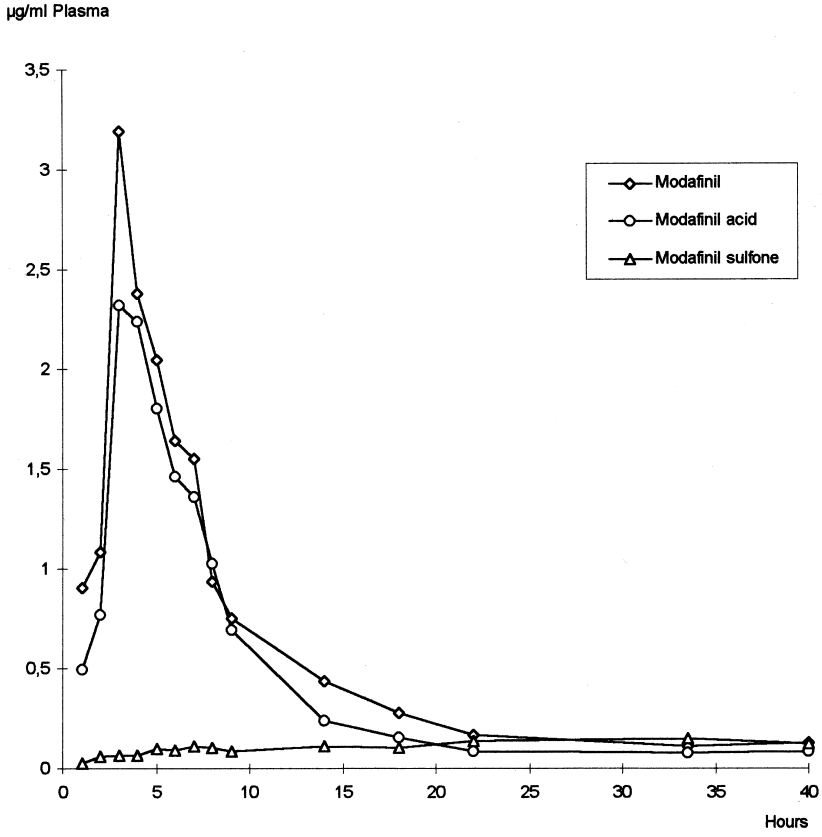


Fig. 3. Plasma concentration–time course of modafinil, modafinil acid and modafinil sulfone following a single oral administration dose of 200 mg of modafinil to a healthy male volunteer.

ters of modafinil ($C_{max}$=3.25 mg/l; $T_{max}$=3 h); are smaller than values previously obtained by Moachon and Matinier [6] ($C_{max}$=5.75 mg/l; $T_{max}$=5 h).

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