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Short communication

LC–ESI-MS determination and pharmacokinetics of adrafinil in rats

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ABSTRACT

A highly sensitive and specific liquid chromatography/tandem mass spectrometric (LC–MS/MS) method for investigating the pharmacokinetics of adrafinil in rats was developed. Rat serum pretreated by solid-phase extraction (SPE) was analyzed by LC–MS/MS with an electrospray ionization (ESI) interface. The mobile phase consisted of acetonitrile:water:acetic acid (35:65:0.1, v/v/v) in an isocratic elution mode pumped at 1.0 ml/min. The analytical column (250 mm × 4.6 mm i.d.) was packed with Kromasil C_{18} material (5.0 μm). The standard curve was linear from 16.5 to 5000 ng/ml. The assay was specific, accurate (R.S.D. < 2.6%), precise and reproducible (within- and between-day precisions R.S.D. <7.0% and <9.0%, respectively). Adrafinil in rat serum was stable over three freeze–thaw cycles at ambient temperature for 6 h. The method had a lower limit of quantitation of 16.5 ng/ml, which offered high sensitivity for the determination of adrafinil in serum. The method was successfully applied to pharmacokinetic studies of adrafinil after an oral administration to rats.

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1. Introduction

Adrafinil (diphenylmethyl)sulfinyl-2 acetohydroxamic acid is one of the psycho stimulants having behavioral activating effects with no adverse side effects, viz. stereotypy and anxiogenesis [1]. It was found to be effective in treating the problems of vigilance, attention, concentration, learning, memory, affective troubles and depressive manifestations [2]. It is generally metabolized to an active form called modafinil (II). Both adrafinil and modafinil serve as alpha-1 adrenergic agonists [3] and increase locomotor activity in mice, rats, monkeys, and dogs [4]. The increase in activity is dose dependent [5,6]. It is important to determine the levels of adrafinil in biological fluids not only for drug development but also for therapeutic monitoring.

A thorough literature search revealed that analytical methods for determination and pharmacokinetics of adrafinil (I) in rat serum were not reported previously. Although, a few analytical methods for the quantification of modafinil (II) in biological samples were available [7–11], but none of them enables the determination of adrafinil (I), modafinil (II) and their acid metabolite (III) in a single chromatographic run. In this paper, for the first time, a simple, rapid, specific, and sensitive HPLC–MS/MS method was developed to determine adrafinil (I) and its two metabolites in rat serum after solid-phase extraction to study its pharmacokinetics in rats. The present method has the advantages of high selectivity, sensitivity, and accuracy. After validation, the proposed method was successfully applied to a pharmacokinetic study of adrafinil following an oral administration of 20 mg/kg adrafinil to rats.

2. Experimental

2.1. Chemicals and reagents

All the reagents were of analytical-grade unless stated otherwise. Glass-distilled and de-ionized water (Nanopure, Barnsted, USA), HPLC-grade acetonitrile (Qualigens Fine-chem. Mumbai, India) and acetic acid (S.D. Fine-chem. Mumbai, India) were used. Adrafinil was purchased from Sigma–Aldrich, USA. II (modafinil) and III (modafinil acid) were synthesized according to reported procedures [12]. Control serum used for calibration curve and validation of the assay was obtained from Wistar rats (Pharmacology Division, Indian Institute of Chemical Technology, Hyderabad, India).

2.2. Animals

Six Wistar rats (200–220 g) were used in the present study. The rats were housed under standard conditions and had ad libitum access to water and standard laboratory diet throughout the experiments. After a single dose by oral administration of 20 mg/kg adrafinil to healthy Wistar rats ($n = 6$), blood samples (1 ml) were

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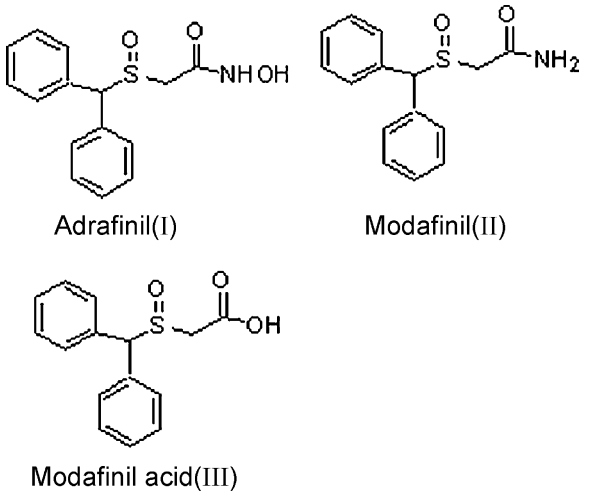

Fig. 1. Chemical structures of adrafinil (I), modafinil (II) and modafinil acid (III).

collected for the determination of adrafinil (I) concentrations. Serial blood samples were collected into the processed test tube at 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 h post-dose. Serum was separated by centrifugation at 4500 × *g* for 5 min and stored frozen at −20 °C. Specimens were thawed and allowed to reach room temperature, and the concentrations of adrafinil (I), II and III were determined from the calibration curve on the same day. Statistical analysis was performed using Microsoft Excel 2000 while pharmacokinetic software, 'Ramkin', based on non-compartment model was used to calculate the [AUC] from the serum drug concentration vs. time profiles [13].

2.3. Liquid chromatography–ESI-tandem spectrometry (LC–ESI-MS/MS)

Liquid chromatographic separation and mass spectrometric detection were performed using a Finnigan Surveyor LC Pump Plus, Finnigan Surveyor Autosampler Plus, Finnigan Surveyor PDA Plus detector and LCQ ion trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA), equipped with an ESI source. The chromatographic separation was achieved on a Kromasil C_{18} (250 mm × 4.6 mm i.d.; particle size 5 μm) analytical column at a temperature of 30 °C. The final mobile phase comprised acetonitrile:water:acetic acid (35:65:0.1, v/v/v). The flow rate was 1.0 ml/min, the total run time was 20 min, the column was maintained at a temperature of 30 °C and the autosampler temperature was 20 °C. The data acquisition was under the control of a Xcalibur software (Thermo Electron Corporation, USA). The mass spectrometer was operated in positive ion mode. Spray voltage was optimized at 5 kV, transfer capillary temperature at 300 °C, sheath gas and auxiliary gas (nitrogen) pressure at 30 and 8 arbitrary units (set by the LCQ software, Thermo Electron Corporation), respectively. Argon was used as collision gas at a pressure of 1.5 mTorr and collision energies used were 20–40 eV throughout the experimental work.

2.4. Preparation of stock, working solution

The stock solutions of I, II and III were prepared by dissolving the analytes in methanol containing 0.05% formic acid to final concentration of 0.51 mg/ml for I, 0.50 mg/ml for II and 0.49 mg/ml for III. The role of formic acid was to generate protonated mass spectra of the analytes. For the assay of serum samples, working solutions were prepared by appropriate dilution of the stock solution with methanol. Separate solutions were prepared for the calibration curve and quality control samples. Standard solutions were obtained by serial dilutions of stock solutions with methanol. All the solutions were protected from light and stored at 4 °C. The samples were stable for at least 9 days under these conditions. The calibration and quality control serum samples were prepared by addition of standard solutions to drug-free serum in volumes not exceeding 2% of the serum. The serum samples were stored in the freezer at −20 °C and thawed at room temperature before processing of the sample.

2.5. Solid-phase extraction

The solid-phase extraction (SPE) cartridges (Oasis HLB, 6 cm^3/200 mg, Waters, USA) were washed with 1 ml of methanol followed by 1 ml of water. 300 μl of serum spiked with the working solutions of I, II and III with suitable concentrations was pipetted to the polypropylene tube and the tube was shaken briefly. The sample was applied to the SPE cartridge and subsequently washed with 1 ml of water. A clean tube was positioned below the SPE cartridge and the compounds were eluted with 1 ml of acetonitrile. The eluent was transferred to an autosampler vial. 20 μl were injected into the chromatographic system. Same procedure was used for method validation and pharmacokinetic studies.

2.6. Calibration curves

The calibration curve was constructed by varying the initial concentration (IC) from 16.5 (I), 21.0 (II) and 25.4 (III) to final 5000 ng/ml to encompass the expected concentrations of measured samples. The concentrations of individual samples were IC, 30, 50, 100, 200, 500, 1000, 2000 and 5000 ng/ml. The calibration curves were obtained by weighted linear regression (weighing factor $1/x^2$) using the Microsoft Excel 2000 software. The suitability of the calibration model was confirmed by back calculating the concentrations of the calibration standards.

Table 1
ESI–LC/MS full-scan and CID product ion spectra for adrafinil (I) and its two metabolites (II and III)

Compound	Collision energy (V)	Mass to charge ratio	
		Precursor ions (*m*/*z*)	Product ions (*m*/*z*)
Adrafinil (I)	30	290 (32%) 233 (10%)	233 (100%), 177 (55%) 177 (100%)
II	20	274 (20%) 167 (10%)	167 (100%) 166 (35%), 165 (100%), 152 (80%)
III	28	275 (15%) 167 (14%)	167 (100%) 166 (30%), 165 (100%), 152 (91%)

('-'%): relative abundance.

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