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Development and validation of a reversed phase liquid chromatographic method for separation and determination of related-substances of modafinil in bulk drugs☆

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Abstract

A reversed-phase high-performance liquid chromatographic (RP-HPLC) method for determination and evaluation of purity of modafinil in bulk drugs using Kromasil C_{18} column with acetonitrile: 0.02 M ammonium acetate as a mobile phase in gradient elution mode at 30 °C and detection at 225 nm using photodiode array detector has been developed. The effects of pH, temperature and the percent of organic modifier on resolution were studied. Related substances, viz, sulphide, sulphoxide, sulphones of the modafinil, acid and ester derivatives, were separated and quantified. The method was found to be simple, rapid, selective and capable of detecting all process related impurities at trace levels in the finished products of modafinil with detection limits of 0.6–2.4 × 10^{-8} g. The method was validated with respect to accuracy, precision, linearity, ruggedness, and limits of detection and quantification. It was found to be suitable not only for monitoring the reactions during the process development but also quality assurance of modafinil.

Keywords: Psycho stimulants; Narcolepsy; Modafinil; Related substances; Reversed-phase HPLC

1. Introduction

Narcolepsy is a disabling, neurological sleeping disorder characterized by chronic steepness and marked disorganization of sleep/awake behavior. A person with narcolepsy nods off while talking, driving, eating and working. It is particularly distressing and potentially dangerous disorder that impairs the quality of life. Patients with obstructive sleep apnea/hypopnea syndrome (OSA/HS), narcolepsy and shift work sleep disorder (SWSD) suffer from excessive sleepness. Armodafinil, the R-enantiomer of modafinil, [2-(1,1-diphenyl methyl sulfinyl) acetamide] is a unique psycho-stimulant α_1- adrenoreceptor agonist that has been recently approved by the food and drug administration (FDA), USA for treatment of narcolepsy. It has shown to be quite effective in improving the wakefulness in such patients. It is currently used to treat patients with daytime sleepness associated with narcolepsy. It may also find usefulness in treatment of attention deficit hyperactivity disorders. Armodafinil at present is undergoing a regulatory review at the FDA for use in improving wakefulness in patients with excessive sleepness associated with narcolepsy, shift work sleep disorder and obstructive sleep apnea/hypopnea syndrome [1–4].

A thorough literature search has revealed that only a few analytical methods are available for determination of modafinil in bulk drugs and pharmaceuticals. Its pharmacokinetic profiles in healthy subjects [5,6] had been well characterized. Detection of modafinil and its major metabolites in equine urine, in human plasma, had been reported [7–10]. Tseng et al. [11] had analysed modafinil by gas chromatography-mass spectrometry. Cass et al. [12], had developed a method for enantioselective assay for (±)-modafinil in human plasma using amylose tris [(*S*)-1-phenylethylcarbamate] chiral stationary phase and elution with acetonitrile: water (25:75 v/v) as a mobile phase. Becue [13] had evaluated the chemical structure of by-products during the synthesis of modafinil by liquid chromatography-mass spectrometry. Drouin and Broquaire [14] had optimized the mobile phase composition for liquid chromatographic separation of optical isomers of modafinil on a chiral-AGP column.

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However, none of these methods address to the problem of separation and determination of all process related impurities, which are most likely to be present in the finished products of modafinil. Further, to best of our knowledge, modafinil is not yet official in any of the pharmacopoeia and no method for determination of its impurities has been reported either in bulk drugs or pharmaceuticals. According to ICH-guidelines [15,16], the limits of related substances and impurities in active ingredient should be ≤0.1%. For finished products, the impurities in daily drug dose ≤2 g/day should be with reporting threshold ≤0.05%, identification threshold ≤0.10%, and qualification threshold ≤0.15%. For daily drug dose ≥2 g/day these should be 0.03, 0.05 and 0.05%, respectively. Thus, there is a great need for analytical methods, which will be helpful to monitor the levels of impurities in the finished products of modafinil during process development. In the present study, separation and determination of sulfide, sulfoxide and sulfone of modafinil, its acid and ester was examined by reversed-phase high-performance liquid chromatography (RP-HPLC) using a C_{18} column connected to photo diode array (PDA) detector at 30 °C temperature.

2. Experimental

2.1. Materials and reagents

All reagents were of analytical-grade unless stated otherwise. Glass-distilled and de-ionized water (Nanopure, Barnsted, USA), HPLC-gradient grade acetonitrile (Qualigens Fine-chem. Mumbai, India) and ammonium acetate (S.D. Fine-chem. Mumbai, India) were used. Process intermediates, viz, sulfide, sulfoxide, sulfone of modafinil, its acid and ethyl ester were synthesized in our laboratory following known procedures [17,18].

2.2. Apparatus

The HPLC system consisting of two LC-20AT pumps, an SPD-M20A diode array detector, a SIL-20AC auto sampler, a DGU-20A_3 degasser and CBM-20A communications bus module (all from Shimadzu, Kyoto, Japan) was used. A reversed-phase Kromasil C_{18} (Hichrome) column (25 cm × 4.6 mm i.d.; particle size 5 μm) was used for separation. The chromatographic and the integrated data were recorded using HP-Vectra (Hewlett Packard, Waldron, Germany) computer system using LC-Solution data acquiring software (Shimadzu, Kyoto, Japan).

2.3. Chromatographic conditions

The mobile phase was 0.02 M ammonium acetate-acetonitrile. The analysis was carried out in a gradient elution mode with 30% acetonitrile at 0 min gradually increased to 60% at 8 min, then increased to 80% at 13 min, from 13 min to 20 min 90% using a flow rate of 1.0 ml/min. at 30 °C. Before delivering into the system the solvent was filtered through 0.45 μm, PTFE filter and degassed under vacuum. The chromatograms were recorded at 225 nm.

Table 1
Gradient program

Time (min)	Solvent A (%)	Solvent B (%)
0.01	70	30
8	40	60
13	20	80
20	10	90
22	70	30
30	70	30

A = 0.02 M ammonium acetate, B = acetonitrile.

2.4. Analytical procedure

Solutions of sulfide, sulfoxide, sulfone of modafinil, its acid and ethyl ester were prepared by dissolving known amounts of the components in the mobile phase. These solutions were adequately diluted to study the accuracy, precision, linearity and limits of detection and quantification (Table 1).

2.5. System suitability

The system suitability was conducted by using 0.1% of all process intermediates spiked to the modafinil and evaluated by making five replicate injections. The system was deemed to be suitable for use as the tailing factor for modafinil was ≤1.2; the resolution was greater than 3.9 or higher. Synthetic mixtures and process samples were analyzed under identical conditions. The quantities of intermediates and assay of modafinil were determined from their respective peak areas (Table 2).

3. Results and discussion

3.1. Synthesis of modafinil and its related substances

Fig. 1 describes the chemical reactions involved in synthesis of modafinil and its related substances. Modafinil acid sulfoxide (I), modafinil acid sulfide (III), modafinil (sulfoxide) (IV), modafinil sulfide (VI), modafinil ester sulfoxide (VII) and modafinil ester sulfide (IX) were synthesized according to the procedure reported by Prisinzano et al. [17]. Initially a mixture of benzhydrol (X) and thioglycolic acid in trifluoroacetic acid (TFA) was stirred at room temperature for 3 h to prepare modafinil acid sulfide (III) as shown in Fig. 1. Substances I, II, VI and IX were synthesized from modafinil acid sulfide (III) following the reactions as shown in Fig. 1. Substances I, IV and VII were synthesized by oxidation of III, VI and IX by H_2O_2 in acetic acid at 40 °C, respectively. A solution of thionyl chloride ($SOCl_2$) in benzene was added dropwise to III in benzene and the resulting mixture was refluxed for 1.5 h at 80 °C. The solvent was removed under reduced pressure to afford a crude orange oil. It was dissolved in dichloromethane (DCM) and added cautiously to a vigorously stirred solution of NH_4OH to form IV. Substance IX was synthesized by refluxing overnight a mixture of substance III in ethanol in presence of H_2SO_4. Sulfones of acid (II), amide (V) and ester (VIII) were synthesized by the over-oxidation of (±)-acid (I), amide (IV) and (±)-ester (VII) respectively at −40 °C with *meta*-chloroperbenzoic