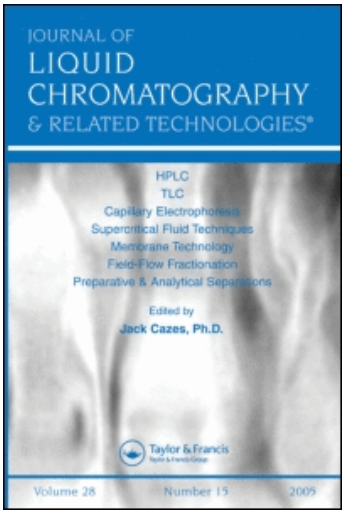

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ASSAY AND STABILITY-INDICATING CAPILLARY ZONE ELECTROPHORETIC METHOD FOR THE DETERMINATION OF MODAFINIL IN BULK AND ITS PHARMACEUTICAL PREPARATIONS

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□ *A simple, sensitive, and cost effective capillary zone electrophoresis (CZE) method for the determination of the novel wake promoting agent, modafinil in pharmaceutical formulations has been developed and validated. The CZE separation was performed using 50 μm i.d × 56 cm fused silica capillary under the following conditions: capillary temperature, 25° C; applied voltage, 25 kV; 20 mM H_3PO_4 – 1 M tris running buffer (pH 9.0); detection wavelength, 225 nm. Phenobarbital was used as the internal standard. The method was validated and showed not only good precision and accuracy but also good robustness. The calibration was linear from 5 to 250 μg mL^{-1}. The accuracy values ranged from 101.6 to 105.3%. The good accuracy values obtained indicate the potential of this method for the determination of the analyte in pharmaceutical formulations. The LOD and LOQ were 1.2 and 3.5 μg mL^{-1}, respectively. The method has been successfully applied to the determination modafinil in pharmaceutical tablet formulations. Excipients present in the tablets and degraded products from different stress conditions did not interfere in the assay.*

Keywords capillary zone electrophoresis, degradation products, forced stress tests, modafinil, pharmaceutical analysis

INTRODUCTION

Modafinil (*rac*-2-[(diphenylmethyl)sulfinyl]acetamide) is a new FDA drug that was developed by Cephalon Inc. and was approved by the FDA in the US in 1998 under the trade name Provigil (Figure 1).[1–3] This relatively new drug possesses stimulating and awaking properties, and has

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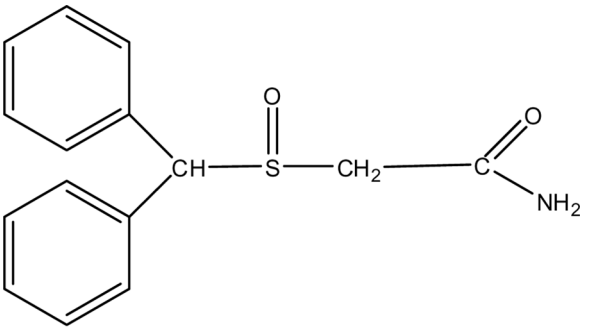
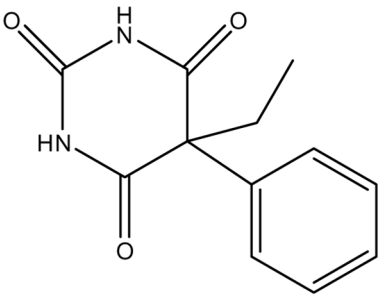


Modafinil (pK_a 14.88)



Phenobarbital (pK_a 7.63)

FIGURE 1 The chemical structures of modafinil and Phenobarbital (internal standard).

been used for treating excessive daytime sleepiness or narcolepsy without interfering with nocturnal sleep.[3–5] The exact mechanism of action in the brain is not yet fully understood, but differs from those of other central nervous system stimulants such as amphetamine or methylphenidate, in that it does not appear to be a dopaminergic agent and presents stimulatory effects of a significantly less general nature. Studies have proposed that modafinil indirectly modulates the release of gamma aminobutyric acid 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.[3,6,7] Various analytical methods are available in the literature for the determination of modafinil in bulk drugs, pharmaceuticals, and in biological fluids. Previous determination of this drug was performed mainly by HPLC in conjunction either with photodiode-array (PDA) or mass spectrometry detectors.[2,4–6,8] A gas chromatography-mass spectrometry (GC-MS) method for the determination of modafinil in human urine was reported.[3]

The capillary electrophoresis (CE) technique is rapidly gaining popularity in the pharmaceutical quality control,[9,10] and has shown great promise

in complementing many conventional methods, especially HPLC. The advantages of short analysis time, small injection volumes (a few nanoliters), and low consumption of solvents render this technique attractive.[11] Recently, general test chapters involving CE has been included in the US,[12] and European Pharmacopoeias.[13]

To the best of our knowledge, the capillary zone electrophoresis (CZE) method for the determination of modafinil has not yet been reported. This study thus describes a simple CZE method for separation and quantification of modafinil in pharmaceutical formulations. The method was further validated as per ICH-Q2A Guidelines.[14] Finally, the developed method was applied to the quality control of pharmaceutical preparations. Phenobarbital (Figure 1) was used as internal standard (IS).

EXPERIMENTAL

Chemical and Reagents

Modafinil standard and modafinil tablets (100 mg modafinil), in addition to phenobarbital standard were kindly donated by HIKMA Pharmaceutical Company, Jordan. Analytical grade methanol was purchased from Merck (Darmstadt, Germany). Ortho phosphoric acid (85%), tris(hydroxymethyl)aminomethane, and sodium hydroxide were purchased from Sigma-Aldrich(USA). Deionised water was produced by a Milli-Q system (Millipore, Bedford, MA, USA), and was used throughout for the preparation of solutions.

Instrumentation and Capillary Electrophoretic Conditions

Separations were conducted on a HP^{3D}CE CZE system (Agilent Technologies, Waldbronn, Germany). The unit was equipped with a photo diode array (PDA) detector. An uncoated fused silica capillary 50 μm i.d × 56 cm, (detection length, 8.5 cm from the outlet end of the capillary) from Agilent Technologies was used. Data acquisition was performed with ChemStation Software. The new capillary was conditioned by flushing for 30 min with 1 M NaOH, 10 min with 0.1 M NaOH, and 15 min with water. Between injections, it was preconditioned for 3 min with 0.1 M NaOH and 3 min with the running buffer prior to each subsequent run. Samples and standards were injected hydrodynamically at 50 mbar for 5 s under the following conditions: voltage, 25 kV (positive polarity); capillary temperature, 25°C; detector wavelength, 225 nm; and the running buffer was 20 mM H_3PO_4-1 M tris solution, pH 9.0. At the end of the day, a final 5 min washing with water was performed. All standards, sample solutions,

the running buffer, and NaOH solution were filtered through an 0.45 μm regenerated cellulose membrane filter (Germany) using Agilent solvent filtration kit.

Preparation of Standard Solutions

Stock solutions of modafinil, and phenobarbital (500 μg mL^{-1}) were prepared by adding 2 mL methanol, then completed to volume with water to the desired concentrations. The modafinil stock solution was used to prepare calibration standards. A fresh stock working solution of IS containing 500 μg mL^{-1} of phenobarbital was also prepared. Working solutions for modafinil was prepared by serially diluting the stock solution with water. All solutions were refrigerated when not in use.

Preparation of Sample Solutions

Ten tablets from each sample were ground into fine powder in a mortar. The powder was quantitatively (equivalent to about 7.5 mg modafinil) transferred into 50 mL volumetric flasks. It was dissolved with the aid of 2 mL of methanol, sonicated for 5 minutes, and then 20 mL water was added, and further sonicated for another 5 minutes. Three mL of phenobarbital solution was added, and was completed to the mark with water. The solution was filtered through a 0.45 μm membrane filter before being subjected to the CZE analysis.

Stress Testing

Stress testing of the drug substance can be used to identify the possible degradation products, provide indication of the stability of the analyte, and can be used to validate the stability and specificity of an analytical method.[14] To investigate the specificity of the proposed method, standard solutions of modafinil was prepared and subjected to four different stress conditions. Aliquots of stock solution of modafinil (2.0 mL of a 1.4 mg mL^{-1}) were transferred into 25 mL volumetric flasks. Each flask was then treated in one of the following ways: (i) heated for 15 h at 75°C, (ii) adding 1000 μL of 1 M hydrochloric acid and heated for 15 h at 75°C, (iii) adding 1000 μL of 1 M sodium hydroxide and heated for 15 h at 75°C, (iv) adding 100 μL of 30% hydrogen peroxide and heated for 15 h at 75°C. A corresponding blank solution was also prepared for each condition. After removing from the stress condition, all samples were cooled to room temperature; the acidic and basic samples were neutralized, and were diluted to a final concentration of 112 μg mL^{-1} analyte and were introduced into the CZE unit.

RESULTS AND DISCUSSION

Optimization of Electrophoretic Conditions

Effect of Buffer pH

Buffer pH is an important parameter in CZE optimization because it affects the ionization of the analytes as well as their electrophoretic mobility.[15]

To study the effect of pH on the migration time and peak width, a constant concentration of 20 mM H_3PO_4-1 M tris solution was investigated in the pH ranging from 7.5 – 10, and was evaluated as running buffer. The results are shown in Figure 2. In general, the migration time decreased as pH decreased but the peak widths were virtually unaffected at pH $\geq$ 8.5. Best results were achieved at pH 9.0, due to good peak shape obtained

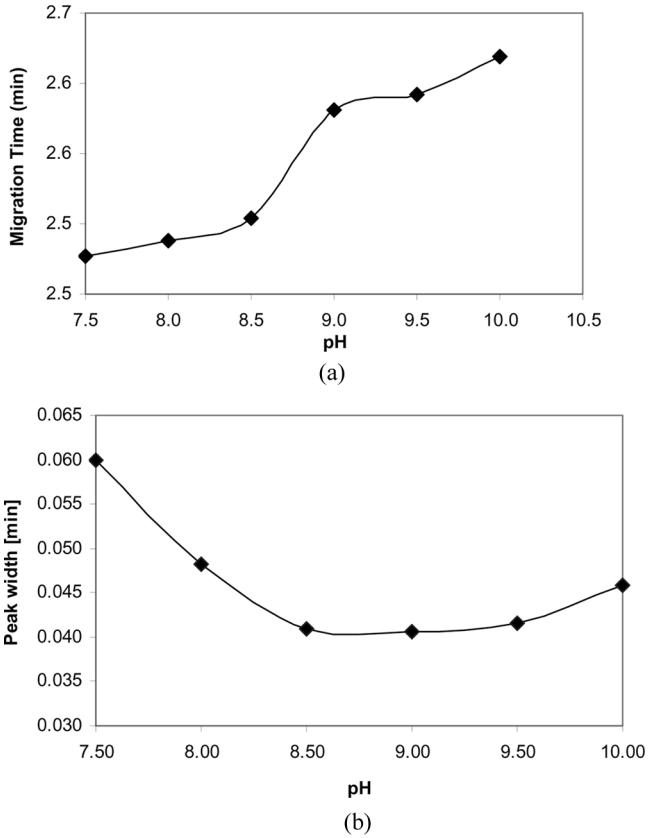


FIGURE 2 Effect of buffer pH on: (a) migration time, (b) peak width. 20 mM H_3PO_4-1 M tris, pH 9.0 buffer solution, voltage: 25 kV, temperature: 25°C, and injection time: 5 s.

compared with other pH values used. Therefore, running buffer with pH 9.0 was chosen for the next measurements.

Effect of Buffer Concentration

The effect of buffer concentration was studied by varying the concentrations of H_3PO_4, from 20 to 100 mM at a constant pH of 9.0. It is known that buffer concentration acts directly on the magnitude of the electroosmotic flow (EOF), a higher buffer concentration giving a lower EOF and vice versa. The recorded current during the separation was high, so more Joule heat was created. If excessive Joule heat cannot be efficiently dissipated, peak efficiency will decrease and the migration time will increase. When 20 mM H_3PO_4 concentration buffer was used, good peak shapes were obtained. The recorded current ($\approx$23 μA) was greatly decreased; under these conditions less Joule heat was generated. A slight increase in the migration time was observed with increasing buffer concentration (data not shown) and 20 mM was chosen as the optimum concentration of the running buffer.

Effect of Applied Voltage

The effect of voltage (15–30 kV) on the migration time of the analyte was also studied. As expected, higher voltage resulted in shorter migration time. At 25 kV, the analysis time was the shortest and the current was not excessive (below 100 μA), so this voltage was selected.

Effect of Capillary Temperature

Capillary temperature can influence the sample stability. The migration velocity of analytes and the number of theoretical plates obtained are proportional to the temperature. The influence of capillary temperature (16–30°C) was evaluated under the chosen running buffer condition. When the temperature started to increase, peak broadening due to the high currents and also decrease in both migration time and resolution occurred.

It is imperative to control Joule heating since this parameter is directly linked to analyte mobility, and stability, as well as the system reproducibility. Decreasing viscosity with temperature is responsible for the nonlinearity of the dependence of velocity on temperature, while an increase in the diffusion coefficient of analyte is responsible for the poorer than expected performance at high temperatures. It can have an impact on the electrophoresis results, primarily through changes in the migration time. Therefore, 25°C was chosen as the working temperature.

Effect of Injection Time

Sample injection time (3−20 s) at 50 mbar was varied to achieve a lower detection limit without affecting the quality of the peak shape and reprodu-

cibility, migration, and resolution. An injection time of 5 s offered best results and was selected for the rest of the studies.

From the above experiments, the adopted conditions for the analysis of modafinil was decided – 20 mM H_3PO_4 – 1 M tris, pH 9.0 as running buffer; injection time, 5 s; applied voltage, 25 kV; capillary temperature, 25°C; and the detection wavelength, 225 nm. Typical eletropherograms obtained under the adopted conditions is shown in Figure 3. The suitability of phenobarbital as internal standard is evident as it is well separated from the analyte peak. All components migrated in less than 4 min.

Method Validation

Calibration Curve, Limits of Detection and Quantitation

Several studies have shown that the use of IS is important to obtain good reproducibility in CZE and chromatographic techniques, in order to compensate for injection errors and minor fluctuations of migration time.[16] In this study, phenobarbital (30 $\mu g\,mL^{-1}$ in all cases) was selected as the IS. The assay of modafinil was validated with respect to linearity, limit of detection (LOD) and quantitation (LOQ), precision, accuracy, robustness, and specificity.

The working solutions containing all two standard compounds were prepared as described above to construct a calibration curve. Each calibration curve contained six different concentrations (5–250 $\mu g\,mL^{-1}$) and was performed in triplicate. A calibration curve with regression equation $y = 0.0461x + 0.6523$ was obtained by plotting the concentration of modafinil in $\mu g\,mL^{-1}$ against the relative corrected peak area. The ratio of relative corrected peak area was chosen, rather than the ratio of peak area, because a lower relative standard deviation (RSD) (2.41%) was obtained. The LOD and LOQ were 1.2 and 3.5 $\mu g\,mL^{-1}$, respectively. LOD was calculated as the amount of the injected sample to yield a signal-to-noise ratio of 3, while the LOQ was taken as the amount of the injected sample to give a signal-to-noise ratio of 10. The method exhibit good linearity ($r^2 = 1.0000$) over a relatively wide concentration range ($5 - 250\,\mu g\,mL^{-1}$). As expected, the sensitivity of the proposed CZE method is slightly inferior compared to the reported HPLC methods,[2,4,5] (LOD ranging from 0.01–0.10 $\mu g\,mL^{-1}$), or GC method,[3] (LOD 0.109 $\mu g\,mL^{-1}$). However, the analysis time of the proposed CE is faster (<4 min compared to ~13 min in the HPLC or ~16 min in the GC).

Precision

The repeatability of the method was examined by ten consecutive injections of 100 $\mu g\,mL^{-1}$ modafinil (the other operating conditions remained

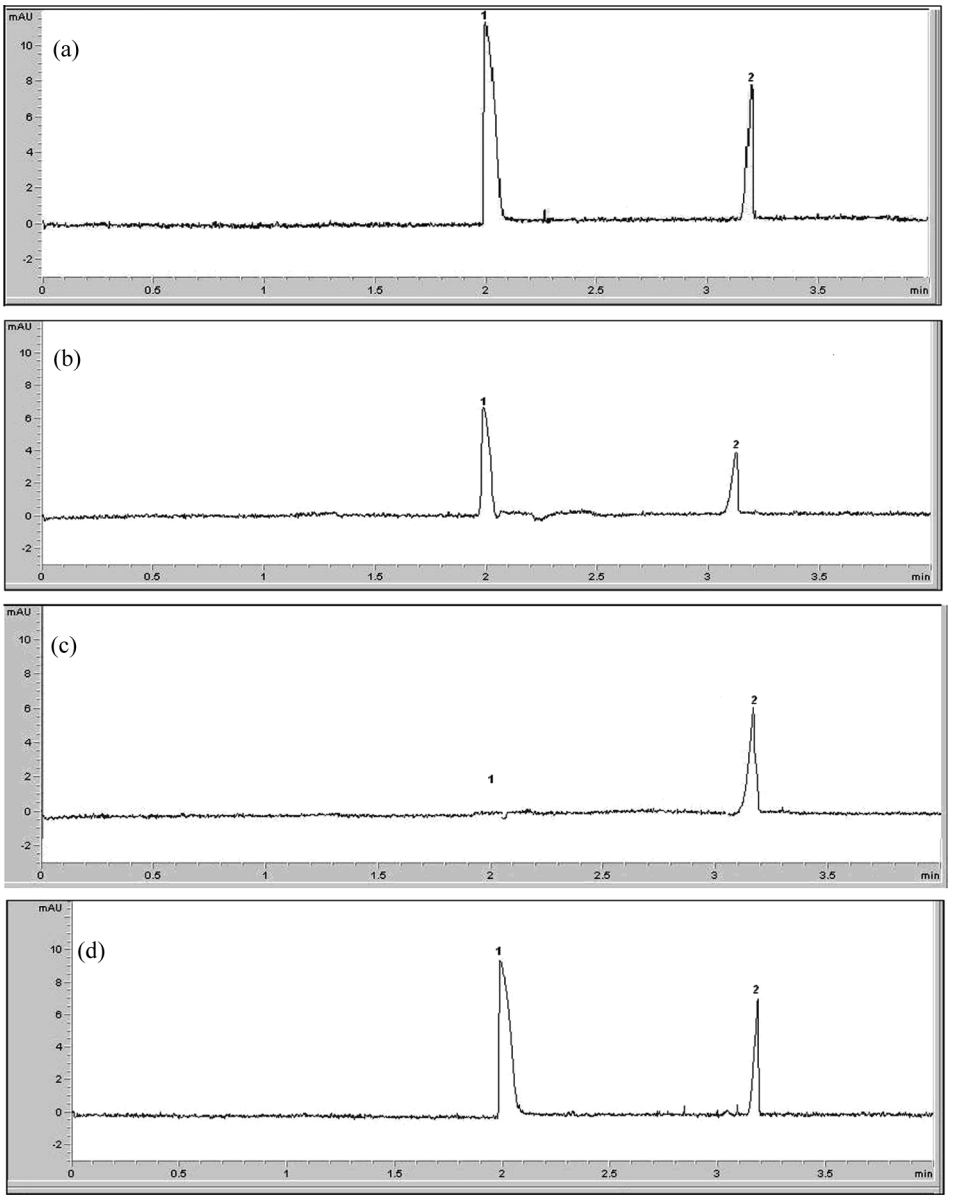


FIGURE 3 Electropherograms of (a) modafinil standard, upon heating at 75°C for 15 h. (b) modafinil standard, containing 1 M HCl, heated at 75°C for 15 h. (c) modafinil standard, containing 1 M NaOH, heated at 75°C for 15 h. (d) modafinil standard, containing 30% H_2O_2, heated at 75°C for 15 h. 1 – modafinil, 2 – Internal standard (phenobarbital). Conditions: 20 mM H_3PO_4-1 M tris, pH 9.0, voltage: 25 kV, temperature: 25°C, and injection time: 5 s.

TABLE 1 Repeatability of Various Parameters Expressed as RSD (%)

Parameter	IS	Modafinil
Migration time	0.35	0.25
Peak area	1.71	2.38
Corrected peak area	1.71	2.44
Ratio of corrected peak area	2.41	
Ratio of peak area	2.60	

IS, internal standard.

identical). The results were evaluated by considering the migration time, peak area, corrected peak area, ratio of corrected peak area, ratio of peak area values of modafinil, and IS. The precision values, with their RSD, are summarized in Table 1.

Intra- and inter-day variations were used to determine the precision of the developed method by analyzing three concentrations (25, 100, and 250 $\mu g\,mL^{-1}$) of standard solutions. The intra-day variation was determined by analyzing the nine replicates on the same day while inter-day variation was conducted over six consecutive days. Intra- and inter day precisions were expressed as RSD ranging from 1.64–3.51 and 1.93–4.41%, respectively (Table 2), indicating the good precision of the newly developed method.

Accuracy

The accuracy of the method was determined by performing recovery tests. An appropriate amount of modafinil tablet powder was weighed and spiked with known amount of the standard and each sample was analyzed in triplicates. The accuracy values ranged from 101.6 to 105.3%. The good accuracy values obtained indicate the potential of this method for the determination of the analyte in pharmaceutical formulations.

Robustness

A robustness test was performed in order to investigate the reliability of the results when the experimental parameters were slightly changed. The

TABLE 2 Intra and Inter-Assay Precision for Modafinil (6 days)

	Modafinil, ($\mu g\,mL^{-1}$)		
	25	100	250
Intra-day (RSD) (%), n = 9	3.51	1.94	1.64
Inter-day (RSD) (%), n = 54	4.41	2.65	1.93

n = no. of introduction, (three preparations for each concentration).

TABLE 3 Determination Modafinil Sample Under Different Conditions Using the CZE Method (n = 6)

	Modafinil	
CZE Running Conditions	Mean ± SD	%RSD
Adopted conditions*	98.33 ± 0.81	0.82
Buffer, pH 8.9	97.30 ± 0.49	0.51
Buffer, pH 9.1	96.71 ± 0.59	0.61
19 mM H_3PO_4-1 M tris Buffer	97.93 ± 0.11	0.11
21 mM H_3PO_4-1 M tris Buffer	97.67 ± 0.53	0.54
24 kV	97.67 ± 0.73	0.74
26 kV	97.56 ± 0.85	0.87

*Please refer to text for details.

variation of the pH of the running buffer by ±0.1, the concentration, and the applied voltage by ±1 did not have a significant effect on the results, indicating the robustness of the developed method (Table 3).

Stress Testing and Specificity

The specificity of the method was evaluated by forcibly degrading the modafinil standard (1.4 mg mL^{-1}) and blanks. Modafinil was found to be stable under elevated temperature and oxidizing conditions. Under the acidic conditions, it was degraded to more than half and was completely degraded under basic conditions (Table 4). Typical electropherograms under these conditions are shown in Figure 3.

No evidence of interference from any excipients in the formulation was found indicating the specificity of the method.

Analysis of Pharmaceutical Formulation

The developed method was applied for the determination of modafinil in pharmaceutical formulation. Good agreement between the proposed method and the manufacturer's claimed values were found for all samples

TABLE 4 Results for the Determination of Modafinil When Subjected to Different Stressed Conditions*

Analyte	Stress Condition	Recovery (%)
Modafinil	Temperature, 75°C	95.1 ± 5.8
	1 M HCl	41.6 ± 3.2
	1 M NaOH	Complete degradation
	30% H_2O_2	90.4 ± 2.7

*All samples were stressed at 75°C for 15 h.

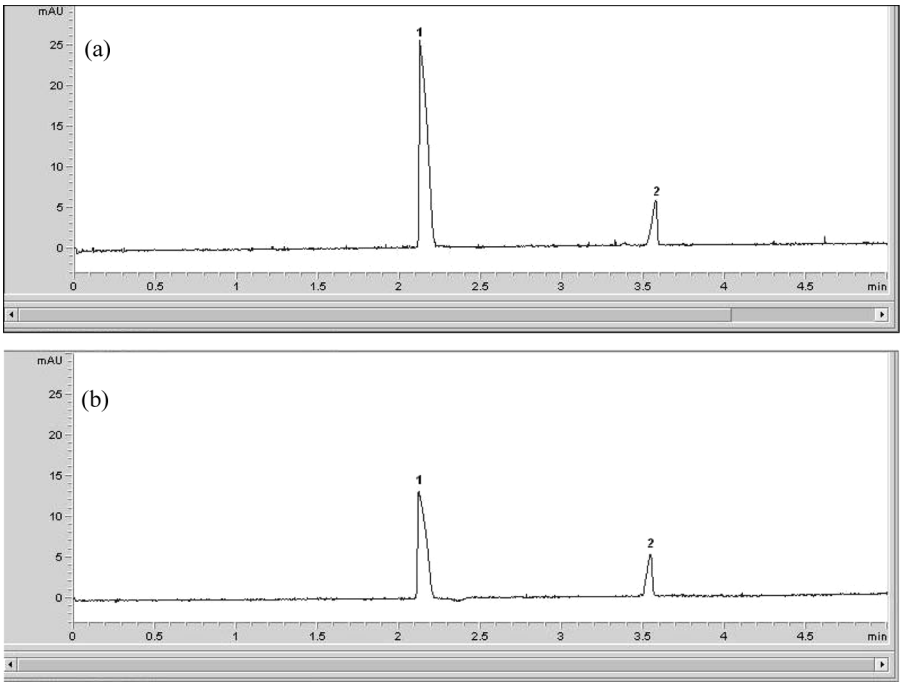


FIGURE 4 Typical electropherograms obtained when operated under the adopted conditions. (a) (250 μg $mL^{-1}$of standard), (b) modafinil tablet. 1 – modafinil, 2 – internal standard (phenobarbital). Conditions: 20 mM H_3PO_4-1 M tris, pH 9.0, voltage: 25 kV, temperature: 25°C, and injection time: 5 s.

(average agreement, 98.3%). Figure 4 shows typical electropherograms of both standard and pharmaceutical formulation samples.

CONCLUSION

The present study describes a rapid, robust, and precise method for the determination of modafinil in pharmaceutical formulation. The stress tests indicate that this drug is stable at elevated temperature (75°C) and under H_2O_2. Under acidic conditions, significant degradation occurs, and was seriously degraded in the presence of NaOH. The degraded products, however, do not interfere in the determination. When compared to HPLC[2,4,5] or GC methods,[3] the proposed method, as expected, exhibits less sensitivity due to the shorter path length of the flow cell, but nevertheless, provides faster analysis time (<4 min compared to ~13 min by HPLC or ~16 min in the GC reports). Higher separation efficiency and minimization of use of solvents are other inherent features of CZE methods. The proposed method exhibits many desirable features such as sensitive, precise, accurate, and is thus recommended to be adopted as quality control protocol in pharmaceutical industries.

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