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Original Research Article

Determination of Venlafaxine and Modafinil in Individual Tablet Dosage Forms using Single RP-HPLC Method

Mohammad Younus[1]*, Md Fasiuddin Arif[2], M Paul Richards[3] and D Bharat Kumar[4]

[1]Chandra Labs, [2]Dr. Reddy's Laboratories Ltd, [3]Malla Reddy College of Pharmacy, Hyderabad Andhra Pradesh India, [4]Vignan Institute of Pharmaceutical Sciences, Nalgonda Dist, Andhra Pradesh, India

For correspondence: E-mail: mdyounus1127@gmail.com; **Tel: +91-8686062421*

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Abstract

Purpose: *To develop a simple and selective isocratic method for the determination of venlafaxine and modafinil in tablet dosage forms.*
Methods: *The compounds were analyzed on Waters symmetry C8 column (250 mm x 4.6 mm i.d, 5µm) using a mobile phase consisting of a mixture of ammonium acetate buffer (pH was adjusted to 4.0 with glacial acetic acid):10 % methanol in acetonitrile, in the ratio of 60:40. The flow rate was 1.0 ml/min and column effluents were monitored at 225 nm. The method was validated according to ICH guidelines.*
Results: *Venlafaxine and modafinil were eluted with retention times of 4.116 min and 6.443 min, respectively. The method was linear in the range of 10 - 50 µg/ml for both venlafaxine and modafinil. The relative standard deviation (%RSD) was < 1 for both drugs while mean recovery values at different concentration levels were within limits. The performance of the method was not changed when small variations in the method were made.*
Conclusion: *The proposed method is accurate, reproducible and low cost, and can be used for the routine analysis of the individual drugs in formulation.*

Keywords: *Venlafaxine, Modafinil, Isocratic method, Validation*

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INTRODUCTION

Venlafaxine is a third-generation, structurally novel phenethylamine bicyclic antidepressant [1]. Venlafaxine inhibits synaptosomal re-uptake of both serotonin and noradrenalin, and it is also a relatively weak inhibitor of dopamine re-uptake [2].

Modafinil, prescribed principally to treat narcolepsy, is undergoing assessment for other neuropsychiatric disorders and medical conditions. The neurochemical substrates of modafinil are unresolved. It has been postulated that modafinil enhances wakefulness by modulating dopamine, norepinephrine, or serotonin transporter activities [3].

There are several methods reported for the determination of venlafaxine in biological fluids [4-7] However, for its determination in drug formulations only two methods have been reported [8-9].

For determination of modafinil in biological fluids [10-14] and formulations [15-16], some methods have been reported. The aim of this present study is to develop a single method for the

determination of venlafaxine and modafinil in their respective dosage forms.

EXPERIMENTAL

Materials and equipment

Waters High Performance Liquid Chromatographic HPLC system equipped with a diode array detector and auto-sampler was used. Waters symmetry C18 column (4.6 mm x 250 mm i.d) was used for separation. Chromatographic and integrated data were recorded using Empower 2 software. All the reagents were of analytical grade unless stated otherwise. Milli Q water, HPLC-grade acetonitrile (Rankem, Mumbai, India), methanol (Rankem, Mumbai, India) and ammonium acetate (AR grade, S.D. Fine Chem, Mumbai, India) were used. All solutions were filtered through 0.45 µm membrane filters procured from Pall Pharma Lab Filtration Pvt Ltd (Mumbai, India).

Preparation of standard solutions

Twenty tablets of venlafaxine were accurately weighed, ground to powder and powder equivalent of 25 mg (1 tablet) of active ingredient was taken into a 50 ml volumetric flask, dissolved in 35 ml of 50% methanol in water (diluent), ultra-sonicated for about 10 min, filtered and the volume made up to the mark with the diluent. From this solution, 0.5 ml of solution was transferred to a 10 ml volumetric flask, 5 ml of diluent was added, sonicated to mix and volume was made up to mark with diluent.

Ten milligrams of modafinil working standard was taken into a 10 ml volumetric flask, dissolved in 4-5 ml of diluent, ultra-sonicated for about 10 min, filtered and the volume made up to the mark with the diluent (Standard Stock). From this solution, 0.2 ml of solution was transferred to 10 ml volumetric flask, 5 ml of diluent was added, sonicated to mix and the volume was made up to mark with diluent.

Preparation of sample solutions

A working standard of venlafaxine (12.5 mg) was taken into a 25 ml volumetric flask, dissolved in 15 ml of diluent, ultra-sonicated for about 10 min, filtered and the volume made up to the mark with the diluent (Standard Stock). From this solution, 0.5 ml of solution was transferred to 10 ml volumetric flask, 5 ml of diluent was added, sonicated to mix and the volume made up to mark with diluent.

Twenty tablets of modafinil were accurately weighed, grounded to powder and powder equivalent of 200 mg (1 tablet) of active ingredient was taken into a 100 ml volumetric flask, dissolved in 70 ml of diluent, ultra-sonicated for about 10 min, filtered and the volume made up to the mark with the diluent. From this solution, 0.1 ml of solution was transferred to a 10 ml volumetric flask, 5 ml of diluent was added, sonicated to mix and the volume made up to mark with diluent.

Preparation of 0.02 M ammonium acetate buffer (pH 4.0)

Ammonium acetate (1.07972 g) was transferred to a 1 L volumetric flask, water (700 ml) was added and sonicated to dissolve and degas, filtered through 0.45µm filter paper and volume was made up to the mark with water. The pH of the resultant solution was adjusted to 4.0 with glacial acetic acid and sonicated for 2 min for proper mixing.

Preparation of 10 % methanol in acetonitrile

Methanol (45 ml) was taken in a 500 ml measuring cylinder, made up to volume with acetonitrile and sonicated for 2 min for proper mixing.

System suitability

Standard solutions of venlafaxine and modafinil were injected six times and chromatograms were recorded. Relative standard deviation (% RSD) of retention times (Rt) and peak areas were calculated. The mean of tailing factor (T. Factor) and theoretical plates (T. Plates) were also calculated.

Specificity

Blank solution (mobile phase), standard solutions, sample solutions and placebo solution (sample solution but excluding active ingredients) were injected separately into the system and chromatograms were recorded.

Precision

Six different samples of both drugs were analyzed and % RSD of assay values was calculated.

Ruggedness (Intermediate precision)

The analysis was performed by a second analyst on Schimadzu HPLC system. The assay of six

different samples was performed and % RSD of assay values was calculated.

Linearity

Solutions in the concentration range of 1 - 50 µg/ml were injected and chromatograms were recorded.

Accuracy

Accuracy of method was measured in terms of % recovery. Sample solutions were prepared at three different concentration levels, i.e., 80, 100 and 120 %. A predetermined amount of standard was added to these solutions and % recovery was determined by assaying the solutions.

Robustness

Slight variations in buffer pH, mobile phase composition, column temperature and flow rate were carried out and standard solution was injected. Six replicates and system suitability tests were performed and the validation parameters indicated above were evaluated.

Solution stability

For mobile phase stability, mobile phase was prepared and stored in a refrigerator. The analysis was performed using freshly prepared sample and standard solutions on the first and second day. For sample solution stability, sample and standard solutions were prepared at specification level and stored in a refrigerator. The analysis was performed using freshly prepared mobile phase on the first and second day.

RESULTS

Kromasil C18 column and mobile phase containing 0.02M ammonium acetate (pH 4.0), 10 % methanol in acetonitrile (60:40) were found to be suitable for analysis of both venlafaxine and modafinil. The other chromatographic conditions optimized were flow rate of 1.0 ml/min, detection wavelength of 225nm, column temperature of 40 ^{0}C, injection volume of 20 ml, diluent methanol:water (50:50, v/v), and a run time of 10 min.

System suitability

To check the system and column performance, the standard solution was injected six times and the following parameters were monitored. System suitability results are shown in Table 1. Tailing factor was < 1.5 for both venlafaxine and modafinil. Theoretical plates were 4500 for venlafaxine and 5500 for modafinil. %RSD of retention time and peak area was < 1 % for both venlafaxine and modafinil.

Table 1: System suitability results

Parameter	Venlafaxine	Modafinil
% RSD of retention time	0.35	0.46
% RSD of peak area	0.61	0.89
Tailing Factor	1.2	1.3
Theoretical Plates	4560	5501

Specificity

The sample and standard chromatograms were identical. There were no peaks in both blank and placebo chromatograms which shows that there was no interference of excipients in the analysis of the drugs. Typical chromatograms are shown in Fig 1.

Precision and intermediate precision

The %RSD values of venlafaxine and modafinil for method precision were 0.71 and 0.58 respectively. But for intermediate precision these are 0.83 and 0.84 for venlafaxine and modafinil respectively.

Linearity

Venlafaxine and modafinil showed linearity in the range 1 - 50 µg/mL, and was represented by the following linear regression equations: Y = 34918X + 1013 (r^2=0.9998), Y = 26759X + 3286 (r^2=0.9998), respectively.

Accuracy

Recoveries of venlafaxine and modafinil were between 98.0 and 102.0 %, indicating good accuracy of the developed method. The results are shown in Table 3.

Robustness

In all cases where deliberate changes were made to the procedure, no significant changes were observed in the results. %RSD of peak area and retention time were < 1 %. The results are shown in Table 4.

Solutions stability

The drug solution and mobile phase were stable up to 48 hours in refrigerated condition. There was not much change in assay value; the results are shown in table no 5.

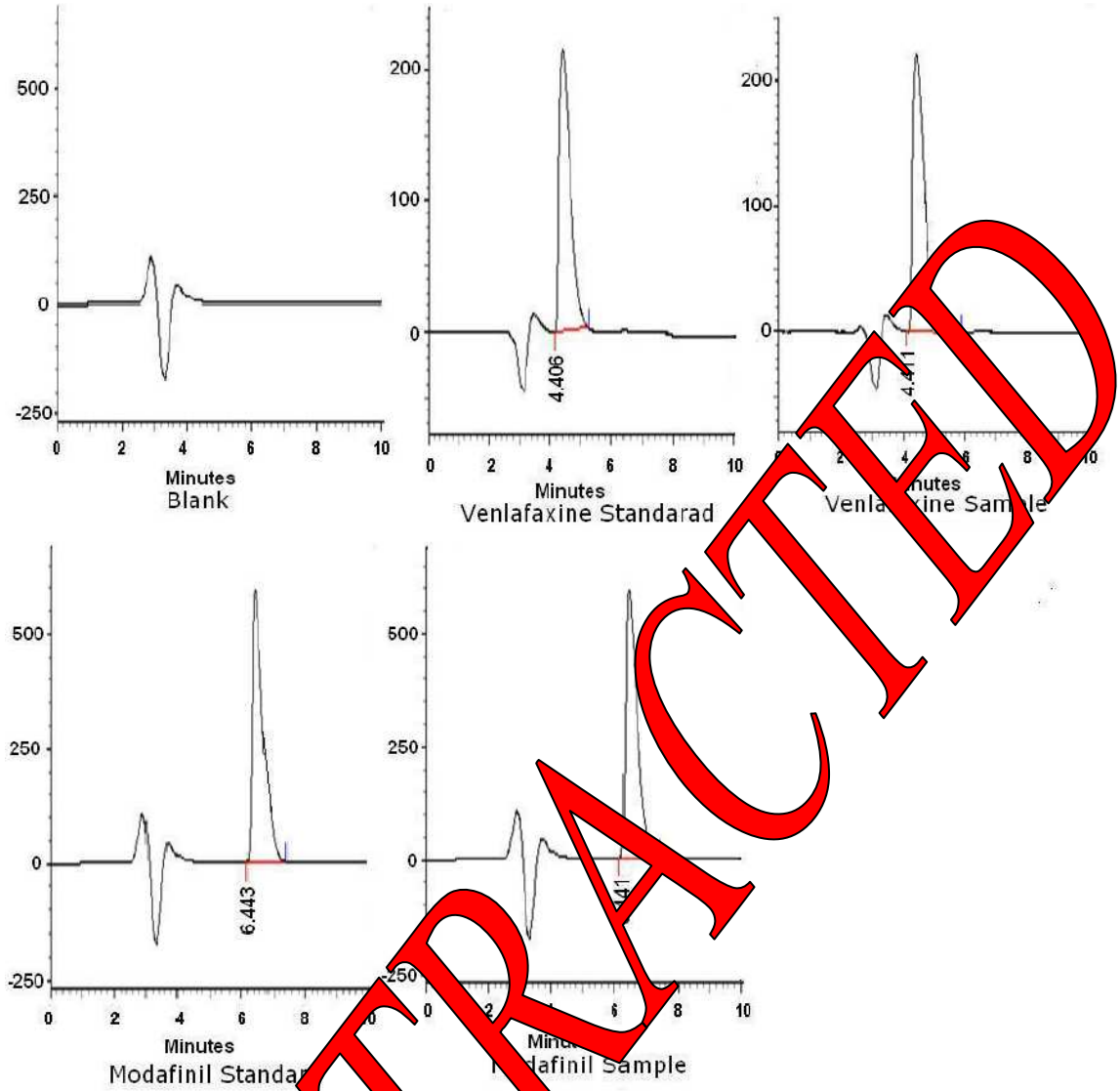

Figure 1: Typical chromatograms

Table 2: Precision and intermediate precision (ruggedness) data

Parameter		Venlafaxine	Modafinil
Precision	Average	99.83	100.04
	%RSD	0.71	0.58
Intermediate Precision	Average	99.67	99.89
	%RSD	0.83	0.84

Table 3: Accuracy (% Recovery) results

% Conc.	Venlafaxine			Modafinil		
	Amount added (µg/ml)	***Amount found (µg/ml)***	***% Recovery***	***Amount added (µg/ml)***	***Amount found (µg/ml)***	***% Recovery***
80	5.00	4.98	99.60	5.00	4.97	99.40
100	10.00	9.92	99.20	10.00	9.95	99.50
120	15.00	14.93	99.53	15.00	14.96	99.73

Table 4: Results of robustness

pH variation	*3.9*		*4.0*		*4.1*	
	Venlafaxine	***Modafinil***	***Venlafaxine***	***Modafinil***	***Venlafaxine***	***Modafinil***
% RSD R_t	0.53	0.41	0.58	0.71	0.65	0.53
% RSD area	0.61	0.47	0.48	0.66	0.69	0.77
T. Factor	1.44	1.11	1.49	1.14	1.53	1.08
T. Plates	4398	5098	4456	5215	4396	5172
Variation of mobile phase composition	***65: 35***		***60:40***		***55:45***	
% RSD R_t	0.38	0.42	0.46	0.62	[illegible]	[illegible]
% RSD area	0.47	0.53	0.34	0.49	[illegible]	[illegible]
T. Factor	1.45	1.09	1.51	1.14	1.63	[illegible]
T. Plates	4231	5341	4837	5169	4657	[illegible]
Temperature variation	***35°C***		***40°C***		***45***	
% RSD R_t	0.58	0.64	0.38	[illegible]	[illegible]	0.53
% RSD area	0.71	0.52	0.63	[illegible]	[illegible]	0.51
T. Factor	1.61	1.09	1.44	[illegible]	[illegible]	1.15
T. Plates	4326	5312	4609	[illegible]	[illegible]	5257
Flow variation	***0.8 ml/min***		***1.0 ml/min***		***[illegible] ml/min***	
% RSD R_t	0.45	0.56	0.39	0.41	[illegible]	0.67
% RSD area	0.79	0.82	0.53	0.36	0.72	0.61
T. Factor	1.51	1.21	1.44	1.15	[illegible]	1.17
T. Plates	4376	5412	4672	5481	[illegible]	5347

Table 5: Solution and mobile phase stability data

Solutions Stability	Assay of Samples			% Variation	
	Initial	1st day	2nd day	1st day	2nd day
Venlafaxine	99.12	99.07	[illegible]37	0.05	0.25
Modafinil	99.33	99.08	98.67	0.25	0.66
Mobile phase stability					
Venlafaxine	99.12	[illegible]	98.75	0.30	0.37
Modafinil	[illegible]9.33	99.12	98.88	0.21	0.45

DISCUSSION

Venlafaxine and modafinil are structurally close and have similar polarities. Both drugs were analysed by reverse phase HPLC on commercial C18 columns, using 0.05M ammonium acetate and 0.05M potassium dihydrogenorthophosphate buffers with organic modifiers, viz, methanol and acetonitrile. Inertsil C18 did not show any selectivity for the compounds. Analysis was tried on a Kromasil C18 (4.6 mm x 250 mm i.d, 5 µm particle size) column, using 0.05M ammonium acetate buffer and methanol as well as methanol/acetonitrile in varying proportions. In methanol alone, modafinil was not eluted, but when acetonitrile was added, modafinil was eluted (as acetonitrile content increased) and the retention times were high. The compounds were analyzed on Waters C18, (4.6 mm x 250 mm i. d, 5µm), using buffers, methanol and acetonitrile in different proportions. The mobile phase containing 0.05M ammonium acetate buffer and 10 % methanol in acetonitrile eluted both compounds in less time, with good peak symmetric properties. The concentration of the organic modifier, buffer pH and column temperature were optimized to separate the two compounds with good resolution in less time.

For efficient analysis of the compounds, various concentrations of acetonitrile were studied. At lower concentrations, it took a longer time for elution of the compounds. Also, there was a slight increase in tailing factor as concentration increased.

Studies were carried out on the effect of buffer pH on tailing factor and retention times. There was a slight increase in tailing of compounds, while the retention time for venlafaxine increased and resolution decreased as buffer pH increased

from 3.0 to 5.0. At pH 4.0, symmetrical peaks with good resolutions were obtained.

The column was maintained at different temperatures ranging from 25 to 50 ˚C. Tailing was reduced with increasing temperature for venlafaxine and a slight increase was observed for modafinil. Retention times decreased slightly with increasing temperature, but the peaks became sharp, and resolution was good for the compounds at 40˚C.

Good symmetrical peaks were obtained with the mobile phase, 0.05M ammonium acetate (pH 4.0): 10 % methanol in acetonitrile (60:40 v/v) on Waters C18 (4.6 mm x 250 mm) column maintained at 40 ˚C. Flow rate was kept at 1.0ml/min. The UV overlaid spectra of both venlafaxine hydrochloride and modafinil showed that both drugs absorb appreciably at 225 nm; hence, 225 nm was selected as the detection wavelength. The retention time of venlafaxine hydrochloride was 4.4 min and that of modafinil 6.3 min. Asymmetric factor for venlafaxine hydrochloride was < 1.5 and for modafinil, it was < 1.2.

Relative standard deviation (% RSD) of retention times (Rt) and peak areas were < 1 and means of tailing factor (> 2), resolution factor (> 2) and theoretical plates (> 2000) were well within the limits, hence the method passed system suitability tests. There was no interference of excipients with the analysis of the drugs. The standard and sample chromatograms were identical, which proves that the method is specific. The mean amount of drugs was 99.[illegible] and 100.04 % for modafinil and venlafaxine, respectively. When analysis was performed by a second analyst on a second system, RSD was < 1 %, , which proves the precision of the method. The method showed good linearity for both venlafaxine and modafinil. The method is robust and unaffected by small variations in test conditions. The method also satisfied stability requirements.

CONCLUSION

A simple and accurate reverse phase HPLC method has been developed for the determination of venlafaxine and modafinil. The method was validated as per ICH guideline in terms of specificity, precision, accuracy, linearity, limit of detection, ruggedness, robustness and solutions and mobile phase stability. A single method can thus be used for the routine analysis of venlafaxine and modafinil in dosage forms.

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