J Fluoresc (2010) 20:821–825
DOI 10.1007/s10895-010-0624-2

ORIGINAL PAPER

Sensitive Spectrofluorimetric Method of Analysis for Venlafaxine in Spiked Rat Plasma and Formulations

Sheikh Shahnawaz · Zaki Siddiqui · Quaisul Hoda

Received: 23 July 2009 / Accepted: 7 February 2010 / Published online: 27 February 2010

Abstract A simple, sensitive, accurate and affordable spectrofluorimetric method was developed and validated for the determination of venlafaxine, both in marketed preparations as well as in spiked rat plasma. Venlafaxine depicted strong native fluorescence property in freshly prepared 0.05 M sulphuric acid. The excitation and emission wavelengths were found to be 237.0 nm and 301.0 respectively. Effect of variations in pH, temperature, concentration, change in molarities of different solvents, and effect of excipients were studied. The calibration graph in case of dosage forms and in spiked plasma was found to be rectilinear in the concentrations of 15–600 ng/ml and 20–650 ng/ml respectively. The intra- day and inter-day accuracy measurements of VEN in formulations ranged from 0.29 to 0.44% and 0.27 to 0.49%, respectively. The intra-day and inter-day accuracy in measurement of VEN in plasma ranged from 0.062 to 2.26% and 0.52 to 2.32%, respectively. The limit of detection (LOD) was found to be 6.0 ng/mL and 4.0 ng/mL in plasma and formulations respectively. The mean recovery of VEN from plasma was 97.46.

S. Shahnawaz (✉) · Q. Hoda
Department of Pharmacy, Lloyd College,
Knowledge park II,
Greater Noida 201306, India
e-mail: sshahnawaz78@yahoo.co.in

Z. Siddiqui
Dreamz College of Pharmacy,
Mandi, Himachal Pradesh, India

Keywords Venlafaxine · Spectrofluorimetric determination · Plasma · Inter and intra- day accuracy

Introduction

Venlafaxine (VEN), chemically designated as, (1-[2-(dimethylamino)-1-(4-methoxyphenyl)ethyl] cyclohexanol) is a second generation antidepressant drug [1]. The pharmacological activity of is attributed to its potent selective serotonin and norepinephrine reuptake inhibition (SSNRI) properties. VEN has demonstrated faster onset of action and better response as compared to the SSNRI antidepressant fluoxetine [2]. VEN has also been successfully used in patients who are not responding to other SSRNIs [3]. The importance of analyzing drugs in body fluids in the last few years is acquiring more importance in antidepressant therapy, especially when metabolic anomalies or low compliance are suspected [4].

Literature survey revealed, many analytical methods exist for the determination of VEN in pure, pharmaceutical preparations as well as in biological fluids. The methods included HPLC coupled with fluorimetric detection [5, 6], spectrophotometric [7-9], electrochemical (coulometric) [10] or mass spectrometric detection [11]. Capillary electrophoresis with UV detection has been used as well [12].

The methods mentioned above are laborious, time consuming and exploit expensive instrumentation HPLC, HPLC–MS, Capillary electrophoresis and coulometric detector.

However, there is no reported spectrofluorimetric method for determination either in dosage forms or in biological fluids. The aim of our work was to develop and validate

spectrofluorimetric method, which is sensitive, simple, reproducible, rapid and affordable.

Materials and methods

Chemicals and reagents

VEN working standard was obtained as gift sample from Torrent labs, Gujarat, India, while tablet Venlift 37.5 mg (Torrent pharmaceuticals) and Ventab XL 37.5 mg (Intas pharmaceuticals) were procured from market. Analytical grade ammonium sulphate, acetonitrile, sodium-hydroxide, sulphuric acid, methanol, sodium dihydrogen orthophosphate, glacial acetic acid, was purchased from Qualigens, Mumbai. Freshly prepared sonicated double distilled water was used throughout the experiment. Rat plasma was stored in deep freezer, at −18 °C, until it was processed for spiking.

Apparatus

Fluorescence intensity was measured on a Shimadzu Spectrofluorophotometer equipped with a xenon-lamp 150 watts (short arc lamp, Ushio inc, Japan) with RF-5301PC software from Shimadzu (Japan). Measurements were taken in a 10-mm path length quartz cell thermostated at a room temperature of 25 °C, with 5 nm bandwidths for excitation and 10 nm for emission monochromator.

Solutions

The stock solution and working standards of VEN were prepared first in methanol. Standard drug solution (100 μg/mL) was prepared, by dissolving 10 mg of VEN in 100 mL volumetric flask, little amount of methanol was added and flask was sonicated for 5 min, then volume was made up to the mark. The further dilutions were made in range of 15–650 ng/mL using 0.05 M freshly prepared sulphuric acid.

Procedures

Preparation of calibration graphs

Two sets of clean and dry, 10 mL volumetric flasks were taken, and separately 1 ml each aliquots equivalent to 0.15–6.5 μg/mL of standard drug preparation and test preparations were added respectively using calibrated micropipettes. Volume was made to the mark with 0.05 M freshly prepared sulphuric acid.

Optimum excitation and emission wavelength were found to be 237.0 nm and 301.0 nm respectively.

Procedures for the plasma

A working standard solution containing 100.0 μg/mL of VEN was prepared. Deep frozen plasma was allowed to thaw at room temperature and was spiked with different quantities of VEN to give a final drug concentration of 20–650 ng/mL, (Table 1). The drug-plasma mixture was incubated at 37.0 °C for 20 min, such that enzymes are activated. To 200 μL of plasma in eppendroff tube, 1.0 mL of acetonitrile and 50 μL of 1.5 M ammonium sulphate were spiked. Tubes were vortexed for 1–2 min and then centrifuged at 5,000 rpm for 5 min. The supernanent organic phase was transferred to test tube and subjected to nitrogen evaporation at 40 °C.

The resultant residue was reconstituted in 2 mL of 0.05 M freshly prepared sulphuric acid. The relative fluorescence intensity was measured at excitation wavelength of 237.0 nm and emission wavelength of 301.0 nm respectively, blank was also treated similarly.

Procedure for tablet formulation

The proposed method was used for analysis of pharmaceutical formulation (Tablet Ventab XL 37.5 mg and Venlift 37.5 mg).A quantity equivalent to 25 mg of VEN was transferred to a clean 100 ml volumetric flask, 25 mL of methanol was added, then volume was made to the mark.

Table 1 Statistical parameters for analysis of VEN

Parameters	Standard preparation	Plasma
Linearity Range	15–600(ng/ml)	20–650 (ng/ml)
LOD (ng/ml)	4.0 (ng/ml)	6.0 (ng/ml)
LOQ (ng/ml)	15.0 (ng/ml)	20.0 (ng/ml)
Slope (b)	4.21	3.38
Intercept (a)	0.021	1.824
Correlation- Coefficient (r)	0.9989	0.9976

The solution was passed through a filter paper to separate the excipients and standard drug concentrations of 15–650 ng/mL were prepared in 0.05 M freshly prepared sulphuric acid.

Method validation

Linearity

The calibration curves were constructed by measuring fluorescence intensities of the analyte against concentrations of the calibration standards. A linear least-square regression analysis was performed for the analyte. The concentrations of the analyte in unknown samples were determined by interpolation.

Accuracy and precision

Accuracy, intra- and inter-day precisions of the method were determined and validated. Six replicate spiked plasma samples were assayed for intra-day and inter-day accuracy at three different concentrations for each analyte. Accuracy was calculated as deviation of the mean from the nominal concentration. Intra-day and inter-day precision were expressed as the relative standard deviation of each calculated concentration.

Recovery

The percentage recovery of VEN in human plasma was calculated by determining average of three replicate measurements.

Specificity

The specificity of the method was determined by adding deliberately some common tablet excipients, such as talc, lactose monohydrate, sodium starch glycollate, starch, hydroxypropyl betacyclodextrin, magnesium stearate to the standard drug preparation and then observing effect on the spectras.

Results and discussion

Method development

The fluorimetric intensity of VEN was determined by scanning a standard drug solution in different solvent systems. VEN standard preparation was scanned in distilled water, 0.01 M, 0.025 M, 0.1 M, sodium hydroxide, 0.01 M, 0.025 M, 0.1 M, acetic acid, methanol, acetonitrile, 0.01 M, 0.025 M, 0.1 M ,phosphate buffer, and 0.01 M, 0.05 M, 0.1 M, sulphuric acid.

VEN depicted a strong native fluorescence property in 0.05 M sulphuric acid; hence it was selected for spectrofluorimetric analysis (Fig. 1). The effect of pH in various solvents and buffers having different molarities were investigated (Fig. 2). Sulphuric acid (0.05 M) having pH of 2.50(±0.01) was found to be the most suitable solvent as far fluorescence intensity and stability of VEN was concerned. The effect of excitation and emission slit width on spectras were seen for 5.0, 10.0, 15.0 nm and it was found that, a slit width of 5.0 nm for excitation and 10.0 nm

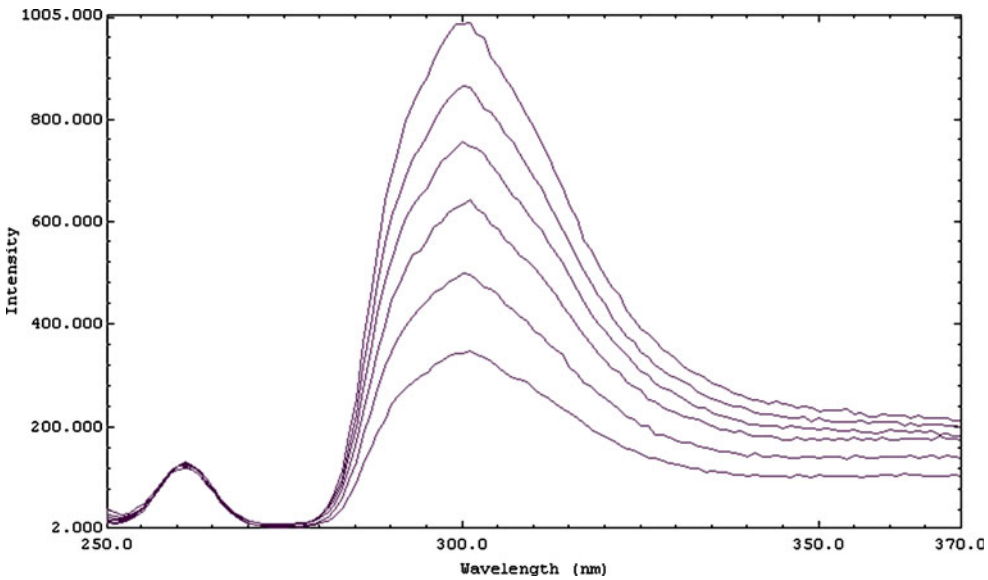

Fig. 1 Emission fluorescence overlaid spectra of VEN 100.0–600.0 ng/mL in 0.05 M sulphuric acid

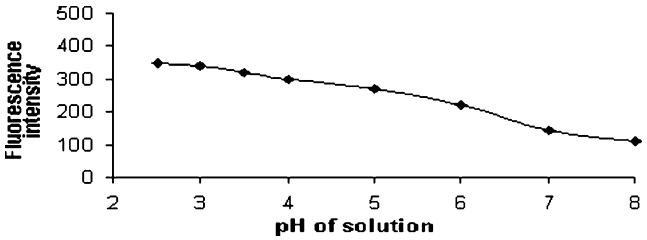

Fig. 2 Effect of pH on fluorescence intensity

for emission wavelength, yielded optimum fluorimetric spectras.

Dipotassium EDTA was used as an anti-coagulant, while as acetonitrile and ammonium sulphate was used as plasma protein precipitant. The advantage of our method was that only 200 μL plasma was required for each analysis.

Method validation

Linearity

The calibration curves of VEN were linear over the concentration range of 20–650 ng/ml for plasma samples and 15–600 ng/mL in case of standard solution. Calibration curve in the linearity range was repeated six times for each concentration and RSD was found to be less than 2.0% indicating method to be highly precise.

Sensitivity

The limit of detection was calculated as 3.3 times signal to noise ratio, using the formula, (LOD=3.3 SDa/b, where SDa is the standard deviation of intercept and b the slope of the regression line). The LOD was found to be 6.0 ng/mL and 4.0 ng/ml for formulation and plasma, respectively.

The limit of quantification was calculated as 10 times signal to noise ratio, using the formula, (LOQ=10 SDa/b, where SDa is the standard deviation of intercept and b the slope of the regression line). The LOQ, in case of formulation and plasma was found to be 15.0 ng/ml and 20.0 ng/ml, respectively.

Table 2 Recovery studies

Amount added (ng/ml)	Amount found (ng/ml)	%Recovery
Standard preparation		
120	116.20	96.83
150	145.50	97.00
180	175.20	97.33
Plasma		
160	155.50	97.19
200	194.30	97.15
240	235.30	98.04

Table 3 Analysis of marketed tablets of VEN, (n=6)

Labeled amount (mg/ml)	Amount found (mg/ml)	% Purity found ± SD
Ventab XL (Intas, 37.5 mg)	36.70±0.05	97.87±0.05
Venlift (Torrent, 37.5 mg)	36.95±0.04	98.53±0.04

Accuracy in formulations and plasma

Both inter-day as well intra-day accuracy were carried out as per international conference on harmonization guidelines, results were highly reproducible. Comparison of results of various published analytical methods revealed that, our method has more or less the same sensitivity as that of HPLC.

The intra- day and inter-day accuracy measurements of VEN in formulations ranged from 0.29 to 0.44% and 0.27 to 0.49%, respectively. The intra-day and inter-day accuracy in measurement of VEN in plasma ranged from, 0.062 to 2.26% and 0.52 to 2.32%, respectively.

Recovery

Recovery studies were carried, by spiking known amount of pure drug 120,150 and 180 ng/ml i.e. (80%, 100% and 120%) of the assay concentration to the preanalysed drug samples; recovery levels were in between 96.83 and 97.33, (Table 2). Recovery results suggest method to be unaffected in the presence of formulation excipients and thus highly accurate.

The mean extraction recoveries of VEN determined at low, medium and high concentrations in plasma, recovery levels were in between 97.15 and 98.04% (Table 2). The RSD of the recoveries were 0.16, 0.21 and 0.28% in 160, 200 and 240 ng/mL plasma standards, respectively. The mean recovery of VEN from plasma was 96.95.

Specificity

The proposed method was used to investigate the effect of commonly present excipients such as talc, lactose monohydrate, Sodium starch glycollate, starch, hydroxypropyl betacyclodextrin, magnesium stearate, no interference was seen. The percentage purity of marketed formulations was calculated by the method and the results are shown in (Table 3)

Conclusion

The proposed method described a validated spectrofluorimetric determination of VEN in presence of excipients. The proposed method was validated with respect to linearity, sensitivity, accuracy, reproducibility and precision. In economic point of view, our method is simple, rapid and economical.

Acknowledgments The author is thankful to Torrent Pharmaceuticals, Gujarat for providing the gift sample of Venlafaxine.

References

1. Tzanakaki M, Guazzelli M, Nimatoudis I, Zissis NP, Smeraldi E, Rizzo F (2000) Int Clin Psychopharmacol 15:29
2. De Nayer A, Geerts S, Ruelens L, Schittecatte M, De Bleeker E, Van Eeckhoutte I, Evrard J-L, Linkowski P, Fossion P, Leyman S, Mignon A (2002) Int J Neuropsychopharmacol 5:115
3. Kaplan EM (2002) Clin Ther 24:1194
4. Capuano B, Crosby IT, Lloyd EJ (2002) Curr Med Chem 9:521
5. Vu RL, Helmeste D, Albers L, Reist C (1997) J Chromatogr B 703:195
6. Waschlager R, Moll W, Knig P, Conca A (2004) Int J Clin Pharmacol Ther 42:724
7. Raut BB, Kolte BL, Deo AA, Bagool MA, Shinde DB (2003) J Liq Chromatogr Relat Technol 26:1297
8. Hicks DR, Wolaniuk D, Russell A, Cavanaugh N, Kraml M (1994) Ther Drug Monit 16:100
9. Duverneuil C, de la Grandmaison GL, de Mazancourt P, Alvarez J-C (2003) Ther Drug Monit 25:565
10. Clement EM, Odontiadis J, Franklin M (1998) J Chromatogr B 705:303
11. Bhatt J, Jangid A, Katesh G, Subbaiah G, Singh S (2005) J Chromatogr B 829:75
12. Fanali S, Rudaz S, Veuthey J-L, Desiderio C (2001) J Chromatogr A 919:195