Review Article

Analytical Methods for Venlaflaxine Hydrochloride and Metabolites Determinations in Different Matrices

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ARTICLE INFO

ABSTRACT

There may be significant variation in stereoisomers related to distribution, bioavailability, metabolism, and excretion. Venlafaxine is a bicyclic phenylethylamine derivative, a unique anti-depressant,which structurally differs from other currently available anti-depressants. Its mechanism of action is believed to be associated with triggering neurotransmitter activity in the CNS. This drug is a racemic mixture of the (−)-*R*-enantiomer and (+)-*S*-enantiomer. *O*-desmethylvenlaflaxine is the major metabolite formed after first pass metabolism with similar potency as parent drug. Two less potent minor metabolites are *N*-desmethylvenlafaxine and *N,O*-didesmethylvenlafaxine. Thus, summarization of all of the analytical methods for the determination of venlaflaxine alone, in combination, or in the presence of other metabolites, is summarized. The keywords used for search were "Analytical methods on Venlaflaxine," "Determination of venlaflaxine," "Determination of venlaflaxine and metabolites," and "Venlaflaxine plasma determinations." Eight spectrophotometry, 26 chromatography, and seven electroanalytical methods were found for the determination of venlaflaxine alone, in formulations, biological matrices, and simultaneous determinations with metabolites and other anti-depressants. Short review of pharmacological activity of venlaflaxine and its metabolites are also presented.

Introduction

Depression is a very common and disabling disease with important social and economic implications. Although many anti-depressant agents are available for the management of this disorder, their efficacy is often limited, because toxic events can be encountered. Their most serious adverse effects include the risk for life-threatening arrhythmias, especially in patients with pre-existing cardiac disease or after overdose administration.[1] Because depression and anxiety disorders are associated with chronic and pervasive psychosocial and occupational dysfunction, they are a significant cause of disability, comparable to the disability associated with chronic illnesses like diabetes, rheumatoid arthritis, and hypertension.[2]

The complex nature of interactions between neurotransmitter systems may limit the accuracy of predictions of an anti-depressant's ability to successfully treat a given patient's symptoms based on its mechanism of action. However, it has been suggested that serotonergic and noradrenergic anti-depressants have differential efficacy in patients with a particular combination of symptoms or subtype of depression; that is, anti-depressants that selectively target 5-HT might be more effective in treating patients with a given symptomatic profile, while those that target NE neurotransmission might be more suited to patients with a different symptomatic profile.[3]

Venlafaxine is a bicyclic phenylethylamine derivative, which is a unique anti-depressant, structurally differs from other currently available anti-depressants.[4] Venlafaxine and its active metabolite, *O*-desmethylvenlafaxine, inhibit the neuronal uptake of norepinephrine, serotonin, and, to a lesser degree, dopamine, but have no monoamine oxidase inhibitory activity and a low affinity for brain musca rinic, cholinergic, histaminergic, or alpha-adrenergic receptors.[5] Hence, it lacks the adverse anti-cholinergic, sedative, and cardiovascular effects of tricyclic anti-depressants.[6] Venlafaxine has an established tolerability and efficacy profile for the treatment of depressive disorders.[5]

It has been proposed that anti-depressants with a dual action of inhibiting the re-uptake of both noradrenalin and serotonin (5-hydroxytryptamine, 5-HT) may be more effective than drugs acting on a single monoamine (e.g. selective serotonin re-uptake inhibitors, SSRIs). Venlaflaxine is the first drug to be marketed that inhibits both noradrenalin and 5-HT re-uptake without actions in other receptors.[7]

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Venlafaxine is available in immediate-release (IR) formulation: ODV half-life is about 10 h (and venlafaxine half-life = 4 h), so this drug can be given in two divided doses. An extended-release (XR) preparation is also available in 37.5, 75, and 150 mg doses: Once-a-day XR dosing achieves bioavailability equivalent to that of twice-a-day dosing with IR formulation. The XR preparation is also associated to a better compliance and demonstrates both anti-depressant and, after 2–3 weeks of treatment, also a good anxiolitic effects.[8] Metabolism of venlafaxine occurs primarily via O-demethylation (mediated by cytochrome P450 [CYP] 2D6) and, to a lesser extent, by N-demethylation (mediated by CYP3A4).[9]

Venlafaxine is unique among anti-depressants in that it down-regulates β-receptors after a single dose, a property which has lead to speculation that venlafaxine may have a more rapid onset of clinic anti-depressant activity than other anti-depressants. Experience with venlafaxine would suggest that multiple mechanisms of action with a tolerance profile, which permits pushing the dosage, may reduce the time to anti-depressant activity.[10]

The prescribing of anti-psychotic and anti-depressant drugs has increased appreciably during the past decade.Example of such a drug is the anti-depressant venlafaxine. This drug is a racemic mixture of the (−)-R-enantiomer and (+)-S-enantiomer. The R-enantiomer inhibits both serotonin and noradrenaline reuptake in vitro, while the S-enantiomer inhibits only serotonin re-uptake.[11]

Venlafaxine undergoes extensive first-pass metabolism to the major O-demethyl metabolite, and 2 minor metabolites. O-demethylvenlafaxine (Referred here O-de-MVX) inhibits noradrenaline and serotonin re-uptake with similar potencies to those of the parent compound[12] and exhibit linear kinetics with an elimination half-life of 5 and 11 hours.[13] Two minor metabolites are N-desmethylvenlafaxine and N,O-didesmethylvenlafaxine (Referred here as N-des-MVX and N,O-dides-MVX) [Figure 1]. These two metabolites may also be considered as pharmacologically active, but they are claimed to be less potent than the parent drug.[11]

Thus, there is no doubt that venlafaxine and its metabolites are clinically important. Hence, there is a need to summarize all of the analytical methods for the determination of this drug in different matrices. The presented review is helpful for researchers engaged in developing new analytical methods and formulations in this field. This review is also useful for the researchers involved in understanding the mechanism of action of each metabolite and their pharmacokinetic and pharmacodynamic relations.

To the best of our knowledge, this is first-time summarization of all of the analytical methods of venlaflaxine along with its metabolites in a single publication is attempted. This review is divided into three sections: Spectrophotometry, chromatography, and electroanalytical methods. Principles, sensitivity (LOD and LOQ), linear range, and applications of each method will be described here.
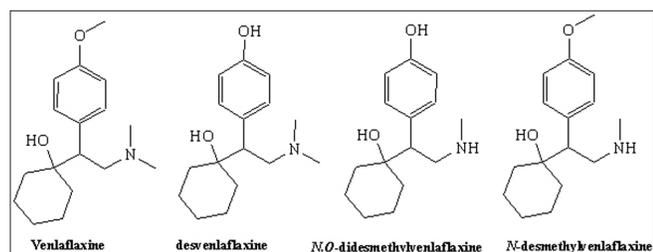

Figure 1: Chemical structures of venlaflaxime and its metabolites

Analytical methods

Venlaflaxine 1-[(1RS)-2-(Dimethylamino)-1-(4-methoxyphenyl) ethyl] cyclohexanol hydrochloride[12] is official in European Pharmacopoeia[14] and British Pharmacopoeia.[15] This is white or almost white powder, freely-soluble in water and in methanol, soluble in anhydrous ethanol, slightly soluble or practically insoluble in acetone. It shows polymorphism.[12]

Spectrophotometry

Nine spectrophotometric methods[16-21] were found. Most of them are not well format and due to lack of scientific evidences, we found difficulty in finding conclusion regarding their sensitivity, applications, and simplicity of the procedure. We found only two publications with reported quantitation and detectionlimits.[18,20] Interesting fact about these publications is the use of buffer (pH 6.8)[16] and absolute alcohol.[21] There are some procedures involving some reactions,[18-20] but they failed to increase sensitivity significantly compared to methods used in distilled water. Method developed by Karani and Pingale[17] is the most economical method reported. Summary of all spectrophotometric methods reported is presented in Table 1.

Chromatography

Twenty-six chromatographic methods[22-48] were found for the determination of VX and its metabolites in different matrices. With quantitation limit of 0.1 ng/ml method developed by Juan et al.[38] for the simultaneous determination and screening of VX, fluoxetine, citalopram, paroxetine in human plasma, is the most sensitive method found. Likewise many chromatography methods are published in the current literature with good sensitivity. All LC-MS/MS determinations are having good sensitivity.[22,31-35,38,40,44,45] GC-NPD[47] and some methods with fluorescence[22,45] detection also have appreciable sensitivity. Five different methods were developed for the determination of VX alone in different pharmaceutical formulations.[23,24,26-28] Count of publications related to determination of VX alone in biological matrices[22,30,33,37,42] also attained same figure. Recent publication is the stability indicating method for the determination of desvenlaflaxine.[41]

Out of these 26 chromatography methods, 10 HPLC-UV methods[23,24,26-28,30,37,39,41,46] were found. Detection wavelengths of all methods found to be near to λ_{max} of VX (near to 225 nm). This shows that pre-column or post-column derivatization methods were not attempted till date. HPLC-UV methods are convenient and more economical than LC-MS methods, but are less sensitive. Quantitation limits of HPLC-UV methods[23,24,26-28,30,37,39,41,46] varies between 0.05 – 600 μg/ml, whereas this range is 0.1 – 5.0 ng/ml for LC-MS systems[22,31-35,38,40,44,45] clearly demonstrated the sensitivity comparison of UV and MS detection.

LC-MS/MS developed by Xiang et al.[22] applied in a pharmacokinetic study of venlafaxine extended release capsule formulation in 20 healthy Chinese male subjects under fed condition. Verapamil was used as internal standard because of its solubility in solvents, similarity between structures, and absence of any chemical reaction with VX. The detection was acquired in the positive ion mode. Nitrogen was used as desolvation gas at a flow rate of 350 L/h. Desolvation temperature was set at 300°C. The ionization source worked at 110°C. Scan time was 0.10s, capillary and cone

Table 1: Summary of different spectrophotometric method

Principle	Detection wavelength	Linear range (μg/ml)	LOD	LOQ	Application	Ref
Drug was dissolved in phosphate buffer of pH 6.8	222 nm	2-26	NM	NM	Tablets	[16]
Drug was dissolved in Distilled water	225.27 nm	4 -24	NM	NM	Tablets and capsules	[17]
Reaction with Picric acid (PA), Chlorophylline coppered trisodium salt (CLPH), alizarin red (AR), ammonium reineickate (RK) reagents	407, 405, 430, and 530 nm for PA, CLPH, AR, and RK, respectively.	8-125, 1.7-23, 9.6-200, and 8.28-160 μg/ml, respectively	0.23, 2.25, 1.26, and 1.66 μg/ml, respectively	0.75, 7.3, 4.2, 5.49 respectively	bulk sample, dosage form and in spiked urine samples	[18]
Green complex of drug with cobalt thiocyanate extracted in nitro benzene	626.4 nm.	10-50	NM	NM	Bulk and tablet dosage forms.	[19]
Reaction with citric acid-acetic anhydride reagent	561.2 nm	8-24	NM	NM		
Third order derivative	285 nm	40-120	1.82	5.49	Tablets	[20]
Sample prepared in ethanol	276 nm	0.5 – 2.5 mg	NM	NM	Tablets	[21]

voltages were 1.2KV and 20V for ESI. Voltages were 19eV and 25eV, respectively, for venlafaxine and verapamil (IS). Quantitation was done using the MRM mode to monitor precursor→production transition of m/z 278→m/z 57 for venlafaxine, and m/z 455→m/z 165 for verapamil. In this study, two formulations were investigated, and both were found to be bioequivalent.

HPLC with coulometric detection method was reported by Clement et al.[29] using paroxetine as internal standard. Paroxetine was selected as an I.S. for two reasons. Firstly, VX and paroxetine are both 5-HT re-uptake inhibitors, and it is unlikely that these would be both administered together to subjects and secondly, paroxetine is well-separated from VX and O-dex-MVX. Authors claimed that that coulometric detection gives more selective measurement of the analytes than UV detection method. VX has a much lower response to electrochemical oxidation than that of O-dex-MVX and paroxetine, consequently a higher voltage setting was required to attain the necessary sensitivity for VX to be measured simultaneously.

Stability indicating high performance liquid chromatographic method[30] was also developed. The sample treated with acid showed an additional peak at a retention time of 4.32 min other than the main peak at a retention time of 5.32 min. It is due to O-demethylation of venlaflaxine of the 4-methoxy group attached to the phenyl ring. This compound being more polar in nature is eluted faster.[30]

Determination of nine anti-depressants in oral fluid and plasma by LC-MS-MS[32] is also available in the current literature including VX. No interferences were found at the retention time of any of the compounds when blank samples or real cases positive to drugs of abuse and other medicines like benzodiazepines were analyzed. Results obtained in this study do not show a good correlation between venlafaxine levels in oral fluid and plasma or the plasmatic-free fraction.

Another similar publication of separation of 11 anti-depressant drugs and 4 of their metabolites through GC-MS[34] is also available. Amitriptyline, citalopram, clomipramine, fluoxetine, fluvoxamine, maprotiline, desmethyl-maprotiline, mirtazapine, desmethyl-mirtazapine, nortriptyline, paroxetine, sertraline, desmethyl-sertraline, venlafaxine, and desmethyl-venlafaxine were investigated in whole blood. In this assay, protriptyline was used as internal standard. This method is useful in routine analysis and for the toxicological analysis during the investigation of different clinical and forensic cases. Solid phase extraction procedure was adopted for extraction of drugs from blood. Since both VX and desmethyl-venlafaxine are tertiary alcohols [Figure 1], dehydrogenation procedure was adopted for derivatization.

Chiral discrimination is frequently encountered in biological systems. The pharmacological and pharmacokinetic differences between drug and enantiomers are often significant.[48] The biological activity of chiral substances often depends upon their stereochemistry, since the living body is a highly chiral environment.[49] Therefore, health and regulatory authorities, such as the US Food and Drug Administration (FDA) defined more strict requirements to patent new racemic drugs, demanding a full documentation of separate pharmacological and pharmacokinetic profiles of the individual enantiomers and their combination.[50]

LC–MS/MS assay for simultaneous determination of venlafaxine (VX) and its active metabolite, O-des-MVX in human plasma was developed using nadolol as an internal standard (IS).[31] Nadolol though belonging to a different class of compounds but with some structural similarity with the analytes was tested as an internal standard. The results were encouraging, as all three compounds had similar chromatographic behavior and were easily extracted from plasma proteins with 0.43% formic acid in acetonitrile. Moreover, there was no significant matrix effect of IS on both the analytes. Use of ammonium trifluoroacetate (1.0 M) in the mobile phase further enhanced the response for both the analytes and IS with low background noise, resulting in higher sensitivity. The most abundant ions found in the product ion mass spectra were m/z 58.1, 58.1 and 254.1 at 35, 45, and 25V collision energy for VX, O-des-MVX, and IS, respectively. Protein precipitation was carried out using ethanol, methanol, acetone, and acetonitrile solvents. The best results were obtained with 0.43% (v/v) formic acid in acetonitrile.

A stereoselective method is described for simultaneous determination of the S- and R-enantiomers of VX, and its three demethylated metabolites in human plasma and whole blood samples[33] are also available in current literature using solid phase extraction techniques. 0.05% formic acid in acetonitrile was added post-column at a flow rate of 0.2 ml/min to increase the sensitivity of the method. The run time was about 35 min for each sample.

This long analysis time meant that a limited number of samples could be processed each day.

HPLC-MS/ESI method for simultaneous determination of VX and its three metabolites *O*-des-MVX, *N*-des-MVX, and *N,O*-dides-MVX in human plasma is developed by Liu *et al*.[44] Gradient elution program was used by varying proportions of two different solvents (solvent A: Ammonium acetate 30 mmol/l, formic acid 2.6 mmol/l, and trifluoroacetic acid 0.13 mmol/l; Solvent B: Acetonitrile). Various anti-depressants (fluoxetine, citalopram, paroxetine, duloxetine, milnacipram, and reboxetine) used in management of depression were evaluated for interference with the assay for VX and its three metabolites.

Another similar publication is simultaneous stereoselective analysis of VX and *O*-des-MVX enantiomers in human plasma by HPLC-ESI/MS using a vancomycin chiral column.[45] Sildenafil was used here as an IS, but the reason is not stated in the manuscript. The possible explanation was that the enantioseparation of VX and *O*-des-MVX on the vancomycin chiral column was the combination of several mechanisms, and thus require more research.

Microorganisms have recently been successfully used as models for drug metabolism studies and for obtaining metabolites that could be developed as new drug entities. One HPLC method is also available for estimation of VX and its metabolites in microbial biotransformation studies. Elute was monitored at 200 nm, probably due to weak absorption of metabolites as compared to parent compound VX, λ_{max} ($\approx$ 225 nm).

Only one research related to stability indicating reversed-phase liquid chromatographic method for the quantitative determination of des-VMS in bulk sample and pharmaceutical dosage form[41] was found. Authors reported formation of 4-[2-(dimethylamino)(1-cyclohexylidine)ethyl)phenol] (*m/z* 246.5), which further degrades into two fragments (201.1 and 58.2 *m/z*).

Spectrophotometric methods are rapid and far more economical than chromatographic methods, but their destructive nature and lack of sensitivity is a huge disadvantage.[51] Increased sensitivity of methods like HPLC-EMS and UPLC-MS/MS is mostly compromised with complicated instrumentations, procedures, and mobile phases. But they are useful in investigation of biological samples.[52]

The summary of reported chromatographic methods ispresented under Table 2.

Electroanalytical methods

Modern electrochemical methods are now sensitive, selective, rapid, and easy techniques applicable to analysis in the pharmaceutical fields, and indeed in most areas of analytical chemistry. They are probably the most versatile of all trace pharmaceutically active compound analysis. Electroanalytical techniques can easily be adopted to solve many problems of pharmaceutical interest with a high degree of accuracy, precision, sensitivity, and selectivity, often in spectacularly reproducible way by employing this approach.[59] Electroanalytical techniques can, in some instances, offer some advantages, among them:(1) Simple sample handling;(2) speed of analysis;(3) high sensitivity; (4) comparable or better accuracy; (5) cheaper instrumentation and lower cost of chemicals used; and (6) limited use of environmentally unfriendly organic solvents.[60]

Seven different electroanalytical methods[15,53-58] were found in literature search. Potentiometric titrimetry method for assay of VX raw material is described in BP.[15] Electroanalytical method with highest sensitivity (LLOQ, 1 ng/ml), which is simultaneous determination of 20 anti-depressants in plasma samples, was carried out by non-aqueous capillary electrophoresis with time of flight mass spectrometry via electrospray ionization.[55]

Carbon nanotubes (CNTs) have started a new era for the development of novel electrode materials due to their amazing structural, mechanical, electrical, and physical properties. They have been successfully used as modifiers to obtain very low detection limits. An application of Nafion/CNT composite film on Glassy carbon electrode (GCE) for the trace determination of VX and des-VMX employing adsorptive stripping differential pulse voltammetry (AdSDPV) is presented by Sanghavi and Srivastav.[54] This method is also one of the most sensitive methods reported till date.

Summary of other electroanalytical methods are described under Table 3.

Future aspects

HPTLC method for the determination of VX alone or its metabolites are not found in literature survey. HPTLC is the only chromatographic method offering the option of presenting the results as an image. Other advantages include simplicity, low costs, parallel analysis of samples, high sample capacity, rapidly obtained results, and possibility ofmultiple detection.[61] The modern HPTLC technique, combined with automated sample application and densitometric scanning, is sensitive and completely reliable, suitable for use in qualitative and quantitative analysis.[62]

The acid-dye method can provide a more sensitive technique for certain amines and quaternary ammonium compounds that absorb weakly in the ultraviolet region. In such methods, addition of an amine in its ionized form to an ionized acidic dye yields a salt (ion-pair) that may be extracted into an organic solvent such as chloroform or dichloromethane. The indicator dye is added in excess, and the pH of the aqueous solution is adjusted (if necessary) to a value where both the amine and dye are in ionized forms. The ion-pair is separated from the excess indicator by extraction into the organic solvent, and the absorbance is measured at the λ_{max} of the indicator in the solvent.[63]

Non-aqueous titrations permit a rapid determination of different types of compounds common in the pharmacy of today: Amines and heterocyclic nitrogen compounds, aminoacids, alkali, and organic salts of weak acids and of hydrogen halides.[64] This is our hypothesis that since venlaflaxine molecule is available in hydrochloride salt, non-aqueous titration method can also be developed in the presence of mercury acetate, and obviously such methods do not require costly equipments and skilled personnel.

Conclusion

Metabolic and regulatory processes mediated by biological systems are sensitive to stereochemistry, and different responses can be often observed when comparing the activities of pair of enantiomers. Thus, regulatory authorities encourage the pharmaceutical industries to provide single enantiomer of drugs, although most of them were commercialized as racemates. Nowadays, the situation has definitely changed, as technical advances permit production of many single enentiomers on a commercial scale. Many enantiomerically pure drugs have successfully reached the market.[50] Thus, there are requirements of literatures including analytical methods of parent drug, its isomers, and metabolites.

Spectrophotometric methods are less expensive, but their

Table 2: Summary of chromatography methods

Method	Mobile Phase/Column	Linear range	LOD	LOQ	Detector	Application	Ref
LC-MS/MS	Inertsil ODS-3 column (50 mm×2.1 mm, 3 μm, Tokyo Japan) an isocratic mobile phase of methanol-10 mmol/L ammonium acetate (85:15,v/v) at a flow rate of 0.30 ml/min.	2-200 ng/ml	NM	NM	MS	Bioavailability and bioequivalence testing of VX with a single-dose administration and multiple-dose administration	[22]
HPLC	Luna C_{18} column (250 mm×4.6 mm) maintained at 35°C. Mobile phase: Ammonium–acetate buffer 32 mM, adjusted to pH 6.8 with phosphoric acid–acetonitrile–methanol (62:30:8, v/v/v), run at a flow rate of 1.0 mL/min	10-70 μg/ml	0.24 μg/ml	0.80 μg/ml	UV, 226 nm	In extended release capsules containing spherical beads and for dissolution studies	[23]
HPLC	XTerra C_8 column (150×4.6 mm i.d., 5 μm) (Waters, USA) at 40°C. Isocratic separation using a mobile phase disodium hydrogen phosphate buffer 40 mM containing triethylamine (pH 6.8) and acetonitrile (75:25, v/v) at flow rate of 1.0 ml/min (runs of 15 min)	0.45-1.05 mg/ml	0.00043 mg/ml	0.00145 mg/ml	UV, 225 nm	Identification of desvenlafaxine in extended-release capsules	[24]
HPLC	Chromolith Performance RP-18e 100 mm×4.6 mm column. Mobile phase methanol:water (35:65, v/v) adjusted to pH 2.5 by phosphoric acid. Flow rate 2 ml/min	1-300 ng/ml for all analytes	NM	1 ng/ml for all analytes	Fluorescence detectore (λ_{ex} 200 nm/λ_{em} 300 nm)	Simultaneous determination with major metabolites O-des-MVX and O, N-dides-MVX in pharmacokinetic studies and therapeutic drug monitoring	[25]
HPLC	A Phenomenex Gemini C_{18}, 5 μm column having 250×4.6 mm i.d. in isocratic mode, mobile phase containing methanol: 0.05 M potassium dihydrogen orthophosphate (70:30, v/v; pH 6.2) was used. Flow rate 1.0 ml/min	0.3-9 μg/ml	100 ng/ml	300 ng/ml	UV, 226 nm	Tablets	[26]
HPLC	A acquity TM BEH column having C_{18}, 100×2.1 mm i.d. in isocratic mode, with mobile phase containing dipotassium hydrogen phosphate: Acetonitrile (30:70 v/v; pH 7.00 with dilute o-phosphoric acid. Flow rate 0.75 ml/min	28-196 μg/ml	6.11 μg/ml	20.33 μg/ml	UV, 227 nm	Tablets	[27]
HPLC	Kromasil C_{18} analytical column (250×4.6 mm i.d., 5 μm particle size) with 0.01 M phosphate buffer (pH 4.5): methanol (40: 60) as a mobile phase, at a flow rate of 1.0 ml/min	48-72 μg/ml	0.075 μg/ml	0.15 μg/ml	UV, 225 nm	Formulations	[28]
HPLC	250×4.6 mm I.D. column (Phenomenex, Macclesfield, UK) packed with a 5 μm Spherisorb ODS/CN [a mixed mode combination of C_{18} (octadecyl) and cyanonitrile material] Mobile phase consisted of 0.05 M potassium phosphate buffer (pH 4.8)–methanol (30:70, v/v) flow-rate 1 ml/min	0–200 ng/ml for both analytes	0.5 ng/ml for both analytes	NM	Coulometric, operating potentials 1, 2 and guard cell were 0.65 V, 0.95 V and 0.98 V.	Plasma profiles of VX and oxydesmethylvenlafaxine	[29]
HPLC	Spherisorb C_8 (4.6×250 mm, 5 μm) column. The mobile phase consisted of acetonitrile:sodium dihydrogen orthophosphate [0.04 M], pH 6.8 (75:25) at a flow rate of 1.5 ml/min.	1–10 μg/ml	150μg/ml	600 μg/ml	UV, 224 nm	Stability indicating (VX)	[30]
LC–MS/MS	Hypurity cyano (50 mm × 4.6mm, 5 μm) column. The mobile phase consisted of 350 ml methanol + 650 ml deionized water + 1.5 ml, 1.0M ammonium trifluoroacetate.	2.0–500 ng/ml for VX and 50-500 ng/ml for O-des-MVX	2.0 ng/ml for both analytes	5.0 ng/ml for both analytes	MS	Bioequivalence study of VX and O-des-MVX	[31]
LC–MS/MS	Sunfire C_{18} IS column (20 mm×2.1mm, 3.5 μm), using a gradient of acetonitrile and ammonium formate (pH 3; 2mM) as mobile phase at a flow rate of 0.4 mL/min. Gradient was applied: 15% acetonitrile until minute 0.5; then, acetonitrile percentage was gradually increased to 50% until minute 4, to increase again to 70% at minute 5.	2 to 500 ng/mL for oral liquid,2 to 1000 ng/mL in the case of plasma samples	NM	2 ng/mL both in oral fluid and in plasma	MS	Simultaneous determination of nine anti-depressants including VX in oral fluid and plasma	[32]
LC/MS-MS	250 mm×2.1 mm Chirobiotic V column. Mobile phase consisted of tetrahydrofuran: 10mM ammonium acetate pH 6 (10:90; v/v)	1–1000 nM for the S- and R enantiomers and -des-MVX, 0.5–500 nM for -des-MVX and -dides-MVX	NM	0.5 nM for VX and -des-MVX, 0.25 nM for -des-MVX and -dides-MVX	MS	Simultaneous determination of the S- and R-enantiomers of VX and its three demethylated metabolites in human plasma and whole blood samples.	[33]

Table 2: Cont.

Method	Mobile Phase/Column	Linear range	LOD	LOQ	Detector	Application	Ref
GC/MS	Cross-linked HP-5MS capillary column (5% phenyl-methylsilicone, 30 m × 0.25 mm i.d., 0.25 μm film thickness) supplied by Agilent Technologies (Illinois, IL, USA). Helium as carrier gas, flow rate of 1.0 mL/min. Injection port and the transfer line at 280 and 300°C, respectively. Initial oven temperature of 100°C was held for 1 min, followed by an increase to 300°C at a rate of 40°C/min with a final hold time of 4 min	5.00–1000 μg/L for both analytes	1.5 μg/L for both analytes	5.00 μg/L for both analytes	MS	Simultaneous determination of 11 anti-depressant drugs including venlaflaxine and des-MVX) in whole blood	[34]
LC-MS-MS	Betasil C_{18} column (3 μm, 100 mm × 3.0 mm) purchased from Thermo electron corporation. A mobile phase consisting of acetonitrile and ammonium acetate (pH 3.5, 5 mM) (75:25, v/v) was delivered with a flow rate of 0.3 ml/min.	3.0–300 and 6–600 ng/ml for VX and des-MVX resp.	NM	3.0 and 6.0 ng/ml, for VX and O-des-MVX	MS	Simultaneous quantification of venlafaxine (VX) and O-des-MVX in human plasma.	[35]
HPLC	Butyl-bonded column (C/E) with mobile phase consisting of acetonitrile and 40 mM phosphate buffer, pH 6.8 (50:50, v/v)	0-2000 ng/ml for both analytes	1 and 5 ng/ml for VX and O-des-MVX resp	NM	Fluorescence detectore (λ_{ex} 276 nm/λ_{em} 598 nm)	Simultaneous measurement of plasma VX and O-des-MVX in human plasma.	[36]
HPLC	5 μm Ascentis C_{18} column (150 mm × 4.6 mm) and mobile phase consisting of 30% acetonitrile in 20 mM potassium phosphate buffer (pH 6.5) delivered isocratically at a flow rate of 1 mL/min	1.05 to 10.5 μg/ml	NM	NM	UV, 228 nm	Gastrointestinal stability of VX *in vitro* in simulated gastric (SGF) and intestinal (SIF) fluids	[37]
HPLC-MS/ESI	Macherey-Nagel C_{18} (250 mm × 4.6 mm, 5 μm, Germany) column, using water (formic acid 0.6%, ammonium acetate: 30 mmol/l)–acetonitrile (35:65, v/v) as mobile phase, with a flow-rate of 0.85 ml/min	5.0–1000.0 ng/ml	MS	0.1 ng/ml	MS	Simultaneous determination and screening of VX in human plasma fluoxetine, citalopram, paroxetine.	[38]
RP-HPLC	SpherisorbÒ S5 C8 analytical column (Waters) (4.6 × 150 mm; 5 μm particle size). Mobile phase acetonitrile–phosphate buffer (6.24×10^{-2} M) (30:70, v/v). Flow rate 1.4 ml/min.	0.2–4 μg/ml for both analytes	NM	50 and 100 ng/ml for VX and -des-MVX resp.	UV, 229 nm	Simultaneous determination of VX and -des-MVX in human plasma	[39]
UPLC-MS/MS	Acquity UPLC TM BEH C_{18} column with 10 mmol/L ammonium acetate and methanol (85:15, v/v) as the mobile phase at a flow rate of 0.30 mL/min. Auto sampler temperature 4°C	0.200–200 ng/mL for both analytes	NM	0.200 ng/mL for both analytes	MS	Simultaneous determine VX and -des-MVX in human plasma, Clinical pharmacokinetic study in healthy male volunteers after oral administration.	[40]
LC-UV	Symmetry Shield column C_{18} (5 μm, 250 mm × 4.6mmi.d.) from Waters, Milford, USA. Mobile phase mixture of 0.2% (v/v) triethylamine in ammonium acetate (0.05 M;pH6.5) and methanol (40:60). Flow rate 1 ml/min.	10–80 μg/mL	5 μg/mL	10 μg/mL	UV, 228 nm	Stability indicating (desvenlafaxine)	[41]
HPLC	Diamonsil column (C_{18}, 250 mm × 4.6 mm I.D., 5 μm) and a mobile phase composed of acetonitrile–phosphate buffer solution (pH 3.0)–triethylamine (33.5:66.5:0.4).	10–800 ngmL^{-1}	<2 ngmL^{-1}	<10 ngmL^{-1}	Fluorescence (λ_{ex} 276 nm, λem 598 nm)	Evaluation of pharmacokinetic profiles in nine healthy volunteers.	[42]
HPLC	Column (C_{18}, 150 × 4.6 mm I.D., 5 μm) and a mobile phase composed of 75% aqueous phosphate buffer containing triethylamine at pH 6.8 and 25% acetonitrile. Mobile phase mixture of acetonitrile (25%, v/v) and a pH 6.8, 40mM phosphate buffer containing 0.25% (v/v) triethylamine (75%, v/v).	1–1000 ngmL^{-1} for both analytes	0.3 ngmL^{-1} for both analytes	1.0 ngmL^{-1} for both analytes	Fluorescence (λ_{ex} 238 nm, λ_{em} 300 nm)	Plasma samples of patients (VX and -des-MVX)	[43]
HPLC-MS/ESI	Thermo BDS HYPERSIL C_{18} (250 mm × 4.6 mm, 5μm, USA) column, using a gradient elution program with solvents constituted of water (ammonium acetate: 30 mmol/l, formic acid 2.6 mmol/l and trifluoroacetic acid 0.13 mmol/l) and acetonitrile (60:40, V/V) at a flow-rate of 1.0 ml/min.	4.0–700, 2.0–900, 3.0–800, 2.0–700 ng/ml for VX, -des-MVX, -des-MVX and dides-MVX resp.	0.4 ng/ml	3.5, 2.2, 2.7 and 1.9 ng/ml	MS	Simultaneous determination of VX and its three metabolites -des-MVX, -des-MVX and dides-MVX in human plasma	[44]
HPLC-ESI/MS	CHRIOBIOTIC V TM (5 μm, 250 mm × 4.6 mm) column with mobile phase constituted of 30 mmol/l ammonium acetate–methanol (15:85, pH 6.0) at a flow rate of 1.0 ml/min	5.0–400 and 4.0–280 ng/ml, for both isomers of VX -des-MVX resp.	1.0 and 1.5 ng/ml for both isomers of VX and -des-MVX resp.	5.0, 5.2, 4.3 and 3.5 ng/ml, for S-VX, R-VX, S- -des-MVX and R -des-MVX resp	MS	Simultaneous stereoselective analysis of VX and its major metabolite -des-MVX enantiomers in human plasma	[45]
HPLC	4.6 X 250 mm SS column (SGE, Japan) Mobile phase consisted of acetonitrile and 0.05 M disodium hydrogen phosphate buffer of pH 3.8 (25:75 v/v) with a flow rate of 1 ml/min.	0.5 – 10 μg/ml	NM	0.05 μg/ml	UV, 200 nm	Estimation of VX in microbial biotransformation studies	[46]

Table 2: Cont.

Method	Mobile Phase/Column	Linear range	LOD	LOQ	Detector	Application	Ref
HS-SPME and GC-NPD*	Alltech EC-5 (30 m × 0.32 mm, 0.25 μm) column. The oven temperature was held at 150°C for 1 min and then increased to 250°C at a rate of 15°C/min, where the temperature was held for 2 min; finally, the temperature achieved to 280 8°C at a rate of 40°C/min. The temperatures of the injector port and the detector were set at 230°C and 300°C, respectively	0.01 - 40 μg/ml	3 ng/ml	10 ng/ml	MS	Post-mortem biological samples	[47]

Table 3: Summary of electroanalytical methods

Method	Principle	Linear range	LOD	LOQ	Application	Ref
Square wave voltammetry	Electrochemical oxidation was studied at a HMDE* electrode over a wide pH range (1.9–10.0) of buffered aqueous solutions using different potential sweep technique. AgCl:Ag:KCl (sat.) reference electrode and a glassy carbon auxiliary electrode	0.25 and 1.9 mg/l	0.124 mg/ml	-	Formulations	[55]
Adsorptive stripping differential pulse voltammetric	Nafion–carbon nanotube-modified glassy carbon electrode (NAF-CNT-GCE) employing this electrode at pH 7.0 in Britton–Robinson buffer (0.05 M) for VF and pH 5.0 in acetate buffer (0.1 M) for DVF	3.81×10^{-8}–6.22 $\times 10^{-5}M$ and 5.33×10^{-8}–3.58 $\times 10^{-5}M$ for VX and des-MVX	1.24×10^{-8} and $2.11 \times 10^{-8}M$ for VX and des-MVX	-	Determination of VX and des-MVX in formulations, urine and blood serum samples.	[56]
Non-aqueous capillary electrophoresis-time of flight mass spectrometry	Background electrolyte: mixture of 60 mM ammonium acetate and 1 M acetic acid in acetonitrile, and water, as well as methanol (100:1:0.5, v/v/v). The spectrometer scanned from m/z 50 to 1000 at 1 scan/s. Capillary voltage set at 4000 V using a capillary exit voltage of 100 V.	0.001–0.5 μg/ml	0.0005 μg/ml	0.001 μg/ml	Simultaneous determination of 20 antidepressants in plasma samples	[57]
Capillary electrophoresis	(Chiral separation) the optimal conditions were: 20 mg:ml of Phosphated γ-Cyclodextrin in a 50 mM Tris-phosphate buffer, set at pH 2.5, 25°Cand 20 kV.	25–500 ng/m for both analytes	25 ng/ml for both analytes	-	simultaneous chiral determination of VX and O-des-MVX	[58]
Non-aqueous capillary electrophoresis	Optimum detection was obtained on a 56 cm (effective length) x 50 μm capillary, using a non-aqueous electrolyte solution system consisting of 7:3 methanol-acetonitrile with 15 mM ammonium acetate, capillary temperature 25°C and hydrodynamic injection,detection at 230 nm.	3.17 – 10.56 μg/ml	0.79 μg/ml	2.39 μg/ml	assay of toxic levels in biological samples (human serum)	[59]
Packed capillary electrochromatography	Mobile phase composed of 100 mM ammonium acetate buffer pH 6/ water/acetonitrile (5:5:90, v/v).	0.05–10 μg/ml for both analytes	0.02 μg/ml both analytes	0.05 μg/ ml for both analytes	chiral separation of VX and metabolite O-des-MVX	[60]

*Hanging mercury drop electrode

sensitivity for the determination of these drugs is questionable. Since VX shows maximum absorption at ≈ 225 nm, this cannot be said to be enriched in chromophore, and thus, its attachment in molecule is another field of research. Sensitivity can be increased in HPLC-UV methods by pre- or post-column derivatization. HPTLC, non-aqueous titration, or acid dye method can further increase the spectrum of analytical methods available. Reactions of stereoisomers may also vary, and this can also form basis for the detection and separation of isomers. Thus, in this way, different analytical methods for the determination of venlaflaxine and its metabolites in various matrices are discussed here.

Acknowledgement

References

1. Harvey AT, Rudolph RL, Preskorn SH. Evidence of the dual mechanisms of action of venlafaxine. Arch Gen Psychiatry 2000;57:503-9.
2. Khalifa M, Daleau P, Turgeon J. Mechanism of sodium channel block by venlafaxine in guinea pig ventricular myocytes. J Pharmacol Exp Ther 1999;291:280-4.
3. Sussman N. SNRIs versus SSRIs: Mechanisms of action in treating depression and painful physical symptoms, primary care companion. J Clin Psychiatry 2003;5 Suppl 7:S19-26.
4. Liu Y, Sun Y, Sun J, Zhao N, Sun M, He Z. Preparation and *in vitro/in vivo* evaluation of sustained-release venlafaxine hydrochloride pellets. Int J Pharm 2012;426:21-8.
5. Troy SM, Dilea C, Martin PT, Rosena AS, Fruncillob RJ, Chianga ST. Bioavailability of once-daily venlafaxine extended release compared with the immediate-release formulation in healthy adult volunteers. Curr Ther Res Clin Exp1997;58:492-503.
6. Makhija SN, Vavia PR. Once daily sustained release tablets of venlafaxine, a novel antidepressant. Eur J Pharm Biopharm 2002;54:9-15.
7. Smith D, Dempster C, Glanville J, Freemantle N, Anderson I. Efficacy and tolerability of venlafaxine compared with selective serotonin reuptake inhibitors and other antidepressants: A meta-analysis. Br J Psychiatry 2002;180:396-404.
8. Biglia N, Torta R, Roagna R, Maggiorotto F, Cacciari F, Ponzone R, *et al*. Evaluation of low-dose venlafaxine hydrochloride for the therapy of hot flushes in breast cancer survivors. Maturitas 2005;52:78-85.
9. Homero de Souza Filho J, Bonifácio FN, Bedor DC, Ramos VL, de Sousa CE, Sardón LL, *et al*. Relative bioavailability of two formulations of venlafaxine extended-release 75-mg capsules in healthy brazilian male volunteers: A single-dose, randomized-sequence, open-label, two-period crossover study in the fasting and fed states. Clin Ther 2010;32:2088-96.
10. Horst WD, Preskorn SH. Mechanisms of action and clinical characteristics of three atypical antidepressants: Venlafaxine, nefazodone, bupropion. J Affect Disord 1998;51:237-54.
11. Karlsson L, Schmitt U, Josefsson M, Carlsson B, Ahlner J, Bengtsson F, *et al*. Blood–brain barrier penetration of the enantiomers of venlafaxine and its metabolites in mice lacking P-glycoprotein. Eur Neuropsychopharmacol 2010;20:632-40.
12. Holliday SM, Benfield P. Venlafaxine: A review of its pharmacology and therapeutic potential in depression. Drugs 1995;49:280-94.
13. Morton WA, Sonne SC, Verga MA. Venlafaxine: A structurally unique and novel antidepressant. Ann Pharmacother 1995;29:387-95.
14. European Pharmacopoeia. Council of Europe. European Pharmacopoeia: Supplement 6.1. 6th ed. Strasbourg: Council of Europe; 2008.
15. The British Pharmacopoeia (BP).England: Stationery Office;2008.
16. Sowmya C, Reddy YP, Kiran Kumar M, Raja MS. Development and validation of spectrophotometric method for the estimation of venlafaxine in bulk and formulations. Int J Chem Sci2011;9:52-8.
17. Karani NA, Pingale P. Analytical method development & validation of venlafaxine hydrochloride in solid dosage forms using UV spectrophotometer. J Pharma Res 2009;2:1246-9.
18. Abdel-Ghani NT, Rizk MS, Mostafa M. Development and validation of extractive spectrophotometric method for determination of venlafaxine hydrochloride in pure solutions, pharmaceutical dosage form and urine samples. Int J Chem Anal Sci 2012;3:1341-7.
19. Raghubabu K, Shanti Swarup L, Kalyanaramu B, Rao MN, Ramdas C. Simple and inexpensive methods development for determination of venlafaxine hydrochloride from its solid dosage forms by visible spectrophotometry. Eur J Chem 2012;9:1645-54.
20. Shirvi VD, Kumar GV, Channabasavaraj KP. Third order derivative spectrophotometric estimation of venlafaxine hydrochloride in bulk and pharmaceutical formulations. Int J Pharm Tech Res 2010;2:700-3.
21. Basaveswara Rao MV, Reddy BC, Srinivasarao T, Prasanthi V. Estimation of venlafaxine in commercial dosage forms using simple and convenient spectrophotometric method. Rasayan J Chem 2009;2:276-9.
22. Xiang J, Feng W, Zhang Z, Yang S. Determination of venlafaxine in human plasma and application in a bioequivalence study by LC-MS/MS. International Conference on Education Technology and Management Engineering Lecture Notes in Information Technology. 2012;16-17:211- 5. Available from: http://www.ier-institute.org/2070-1918/lnit17/v17/211.pdf. [Last assessed on 2012 Oct 22].
23. Bernardi LS, Oliveira PR, Murakami FS, Borgmann SH, Arend MZ, Cardoso SG. Development and validation of a stability-indicating lc method for the determination of venlafaxine in extended-release capsules and dissolution kinetic studies. J Chromatogr Sci 2009;47:770-6.
24. Carneiro WJ, Andrade CH, Braga RC, de Oliveira V. Identification of desvenlafaxine, the major active metabolite of venlafaxine, in extended-release capsules. Revista Eletronica de Farmacia 2010;4:39-53.
25. Ardakani YH, Foroumadi A, Rouini MR. Development and validation of a rapid HPLC- fluorescence method for simultaneous determination of venlafaxine and its major metabolites in human plasma. Daru 2010;18:97-102.
26. Baldania SL, Bhatt KK, Mehta RS, Shah DA, Gandhi TR. RP-HPLC Estimation of Venlafaxine Hydrochloride in Tablet Dosage Forms. Indian J Pharm Sci 2008;70:124-8.
27. Chhalotiya UK, Patel HB, Bhatt KK. Development and validation of an ultra performance liquid chromatography method for venlafaxine hydrochloride in bulk and capsule dosage form. Indian J Pharm Sci 2010;72:814-8.
28. Kaur J, Srinivasan KK, Joseph A, Gupta A, Singh Y, Srinivas KS, *et al*. Development and validation of stability indicating method for the quantitative determination of venlafaxine hydrochloride in extended release formulation using high performance liquid chromatography. J Pharm Bioallied Sci 2010;2:22-6.
29. Clement EM, Odontiadis J, Franklin M. Simultaneous measurement of venlafaxine and its major metabolite, oxydesmethylvenlafaxine, in human plasma by high-performancenliquid chromatography with coulometric detection and utilization solid-phase extraction. J Chromatogr B Biomed Sci Appl 1998;705:303-8.
30. Makhija SN, Vavia PR. Stability indicating LC method for the estimation of venlafaxine in pharmaceutical formulations. J Pharm Biomed Anal 2002;28:1055-9.
31. Patel BN, Sharma N, Sanyal M, Shrivastav PS. Liquid hromatography tandem mass spectrometry assay for the simultaneous determination of venlafaxine and O- desmethylvenlafaxine in human plasma and its application to a bioequivalence study. J Pharm Biomed Anal 2008;47:603-11.
32. de Castro A, Concheiro M, Quintela O, Cruz A, López-Rivadulla M. LC–MS/MS method for the determination of nine antidepressants and some of their main metabolites in oral fluid and plasma study of correlation between venlafaxine concentrations in both matrices. J Pharm Biomed Anal 2008;48:183-93.
33. Kingbäck M, Josefsson M, Karlsson L, Ahlner J, Bengtsson F, Kugelberg FC, *et al*. Stereoselective determination of venlafaxine and its three demethylated metabolites in human plasma and whole blood by liquid chromatography with electrospray tandem mass

spectrometric detection and solid phase extraction. J Pharm Biomed Anal 2010;53:583-90.

34. Papoutsis I, Khraiwesh A, Nikolaou P, Pistos C, Spiliopoulou C, Athanaselis S. A fully validated method for the simultaneous determination of 11 antidepressant drugs in whole blood by gas chromatography–mass spectrometry. J Pharm Biomed Anal 2012;70:557-62.

35. Bhatt J, Jangid A, Venkatesh G, Subbaiah G, Singh S. Liquid chromatography–tandem mass spectrometry (LC–MS–MS) method for simultaneous determination of venlafaxine and its active metabolite *O*-desmethyl venlafaxine in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci 2005;829:75-81.

36. Vu RL, Helmeste D, Albers L, Reist C. Rapid determination of venlafaxine and *O*-desmethylvenlafaxine in human plasma by high-performance liquid chromatography with fluorimetric detection. J Chromatogr B Biomed Sci Appl 1997;703:195-201.

37. Asafu-Adjaye EB, Faustino PJ, Tawakkul MA, Anderson LW, Yu LX, Kwon H, *et al*. Validation and application of a stability-indicating HPLC method for the *in vitro* determination of gastric and intestinal stability of venlafaxine. J Pharm Biomed Anal 2007;43:1854-9.

38. Juan H, Zhiling Z, Huande L. Simultaneous determination of fluoxetine, citalopram, paroxetine, venlafaxine in plasma by high performance liquid chromatography–electrospray ionization mass spectrometry (HPLC–MS/ESI). J Chromatogr B Analyt Technol Biomed Life Sci 2005;820:33-9.

39. Matoga M, Pehourcq F, Titier K, Dumora F, Jarry C. Rapid high-performance liquid chromatographic measurement of venlafaxine and *O*-desmethylvenlafaxine in human plasma Application to management of acute intoxications. J Chromatogr B Biomed Sci Appl 2001;760:213-8.

40. Qin F, Li N, Qin T, Zhang Y, Li F. Simultaneous quantification of venlafaxine and *O*-desmethylvenlafaxine in human plasma by ultra performance liquid chromatography–tandem mass spectrometry and its application in a pharmacokinetic study. J Chromatogr B Analyt Technol Biomed Life Sci 2010;878:689-94.

41. Pawara SM, Khatalb LD, Gabhea SY, Dhaneshwara SR. LC–UV and LC–MS evaluation of stress degradation behavior of desvenlafaxine. J Pharm Anal2012;2:264-71.

42. Qin XY, Meng J, Li XY, Zhou J, Sun XL, Wen AD. Determination of venlafaxine in human plasma by high-performance liquid chromatography using cloud-point extraction and spectrofluorimetric detection. J Chromatogr B Analyt Technol Biomed Life Sci 2008;872:38-42.

43. Mandrioli R, Mercolini L, Cesta R, Fanali S, Amore M, Raggi MA. Analysis of the second generation antidepressant venlafaxine and its main active metabolite *O*-desmethylvenlafaxine in human plasma by HPLC with spectrofluorimetric detection. J Chromatogr B Analyt Technol Biomed Life Sci 2007;856:88-94.

44. Liu W, Cai HL, Li HD. High performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-MS/ESI) method for simultaneous determination of venlafaxine and its three metabolites in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci 2007;850:405-11.

45. Liu W, Wang F, Li HD. Simultaneous stereoselective analysis of venlafaxine and *O*-desmethylvenlafaxine enantiomers in human plasma by HPLC-ESI/MS using a vancomycin chiral column. J Chromatogr B Analyt Technol Biomed Life Sci 2007;850:183-9.

46. Vidyavathi M, Krishna DR, Prasad KV, Vidyasagar J. Rapid HPLC Determination of Venlafaxine in Microbial Biotransformation Studies. Curr Trends Biotechnol Pharm 2009;3:64-70.

47. Mastrogianni O, Theodoridis G, Spagou K, Violante D, Henriques T, Pouliopoulos A, *et al*. Determination of venlafaxine in post-mortem whole blood by HS-SPME and GC-NPD. Forensic Sci Int 2012;215:105-9.

48. Shaw CJ, Guzman NA. Application of capillary electrophoresis technology in pharmaceutical industry. In: Ohannesian L, Streete A, editors. Hand book of pharmaceutical Analysis. New York: Marcel DekkerInc.; 2002.p. 321.

49. Chamsaz M, Asadpour S, Yazdi AS, Ghasemi J. High-performance liquid chromatographic enantioseparation of drugs containing multiple chiral centers on chiral stationary phases. J Iran Chem Res 2009;2:1-21.

50. Maier NM, Franco P, Lindner W. Separation of enantiomers: Needs, challenges, perspectives. J Chromatogr A 2001;906:3-33.

51. Shrivastava A, Gupta VB. A review on various analytical methods on some alpha adrenergic antagonists. Curr Pharma Anal 2011;7:27-41

52. Shrivastava A, Gupta VB. Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Prazosin, Terazosin, and Doxazosin in Pharmaceutical Formulations. Sci Pharm 2012;80:619-31.

53. Lima JL, Loo DV, Delerue-Matos C, da Silva AS. Electrochemical behaviour of Venlafaxine and its determination in pharmaceutical products using square wave voltammetry. Farmaco 1999;54:145-8.

54. Sanghavi BJ, Srivastava AK. Adsorptive stripping differential pulse voltammetric determination of venlafaxine and desvenlafaxine employing Nafion–carbon nanotube composite glassy carbon electrode. Electrochim Acta 2011;56:4188-96.

55. Sasajima Y, Lim LW, Takeuchi T, Suenami K, Sato K, Takekoshi Y. Simultaneous determination of antidepressants by non-aqueous capillary electrophoresis-time of flight mass spectrometry. J Chromatogr A 2010;1217:7598-604.

56. Rudaz S, Stella C, Balant-Gorgia AE, Fanali S, Veuthey JL. Simultaneous stereoselective analysis of venlafaxine and *O*-desmethylvenlafaxine enantiomers in clinical samples by capillary electrophoresis using charged cyclodextrins. J Pharm Biomed Anal 2000;23:10715.

57. Purdel NC, Bălălău D, Ilie M, Arama CC. Determination of venlafaxine toxic levels from human serum by non-aqueous capillary electrophoresis. Farmacia 2010;58:62-9.

58. Desiderio C, Aturki Z, Fanali S. Use of vancomycin silica stationary phase in packed capillary electrochromatography II. Enantiomer separation of venlafaxine and *O*-desmethylvenlafaxine in human plasma. J Chromatogr A 2001;919:195-203.

59. Dogan-Topal B, Ozkan SA, Uslu B. The analytical applications of square wave voltammetry on pharmaceutical analysis. Open Chem Biomed Methods J 2010;3:56-73.

60. Zuman P. Principles of applications of polarography and voltammetry in the analysis of drugs. FABAD J Pharm Sci 2006;31:97-115.

61. Shrivastava A, Saxena P, Gupta VB. Spectrophotometric estimation of Tamsulosin Hydrochloride by acid dye method. Pharm Methods 2011;2:53-60.

62. Shrivastava A, Gupta VB. HPLC: Isocratic or gradient elution and assessment of linearity in analytical methods. J Adv Sci Res 2012;3:12-20.

63. Attimarad M, Mueen Ahmed KK, Aldhubaib BE, Harsha S. High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery. Pharm Methods 2011;2:71-5.

64. Ekeblad P, Erne K. Non-aqueous acid-base titrations in pharmaceutical analysis. J Pharm Pharm 1954;6:433-9.