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Sensitive Determination of Venlafaxine in Urine Samples by Using HPLC-DAD System After Fabric Phase Sorptive Extraction

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ABSTRACT

This study is focused on developing a sensitive analytical method for Venlafaxine molecule which is antidepressant drug belonging to the serotonin-noradrenaline reuptake inhibitors (SNRI) group. With this study, a separation and preconcentration method based on fabric phase sorptive extraction(FPSE) method was developed for low levels of Venlafaxine in urine samples. Experimental variables such as pH, ionic strength, desorption solvent, and other parameters were studied and optimized step by step. The linearity of method under optimized conditions is in the range of 15.00-750.00 ng mL^{-1} while limit of detection is 4.28 ng $mL^{-1.}$ The relative standard deviation (RSD %) obtained from model solutions containing 300 ng mL^{-1} of Venlafaxine was lower than 3.1 % and pre-concentration factor was calculated as 62.50 for target molecule. In order to test accuracy of the method, recovery tests were carried out by means of spiked urine samples. As a result of recovery tests, quantative values were obtained in the range of 97.5-104.2 % successively.

Keywords: HPLC, Fabric phase sorptive extraction, Venlafaxine, Analytical method development.

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Introduction

Antidepressant drugs are widely and effectively used in the treatment of especially depression many mood disorders, psychotic disorders and substance abuse disorder mood disorders. Its use has become widespread in recent years and is an antidepressant belonging to the serotonin-noradrenaline reuptake inhibitors (SNRI) group. In addition to low doses of serotonin and noradrenaline, high doses of serotonin and noradrenaline, they inhibit dopamine reuptake. Side effects can include nausea-vomiting, headache, dizziness, constipation, anorexia, insomnia, dermatitis, irritability, and blurred vision [1,2]. Venlafaxine was first released in 1993 by Wyeth as a quick release tablet for use per more than one doses in day[3]. Venlafaxine, which is a tertiary, bicyclic compound in the phenylethylamine structure, differs from other antidepressants as chemical structure. It is racemic compound composed of R and S enantiomers combined in equal amounts [4]. It is also used in sleep, anxiety and eating disorders, pain syndromes, arrhythmia and some immune dysfunction cures [5].

Detection of antidepressant drugs in biological samples is used in various fields, especially toxicological and therapeutic drug monitoring. Due to the complexity of samples and low concentration levels of drugs, sample preparation is requisite for the development of chromatographic methods [6]. Separation and pre-concentration methods are used for target analyte by collect smaller volume than its volume so level that can be analysed by increase concentration of target analyte. Thus, concentration of target analyte increased and exist at measurable level. The separation process is done by removing the impurities and the other matrix components that may have an adverse effect on the analysis of the analyte or cause contamination of the analyser[7,8]. Conventional sample preparation techniques such as solid phase extraction (SPE) are still among the most frequently used for analytical sample preparation but, these techniques prone to error during extraction steps such as time consuming, exhausting, multistep, often toxic and hazardous organic solvents used in large volumes, solvent evaporation and sample dilution in the appropriate solvent [9]. So, solid phase micro extraction(SPME) technical are more popular in the last decades because they minimize solvent consumptions and total analysis time. The solid phase extraction method is based on collecting the analyte on a solid phase material. Although these method has superior advantage according to exist method, it has some lacking. Thus, researchers go on to study new material and led to the find of several microextraction techniques [10]. As a result of the evaluation of different micro-extraction techniques, it was revealed that the deficiency in all these techniques was caused by the coating technology used for fixed the sorbent on the substrate surface and the physical format

of the extraction systems determining PCSA [11]. Considering all these problems, a new, promising and wide-use extraction technique, called the fabric phase sorptive extraction method (FPSE), was developed by Kabir and Furton in 2014[12,13]. FPSE method provides a very efficient, green sample preparation technique by successfully integrating the advantages of sol-gel derivative sorbents used in microextraction and various fabric phases. Low sorbent capacity and long sample preparation time, two main limitations of absorbent extraction techniques, have been handled by fabric phase extraction [14].

In this work a sensitive determination method is developed for low concentrations of Venlafaxine molecule by using the HPLC-DAD system after the application of FPSE. The developed method was applied to simulated urine and normal urine samples after optimization procedures was completed successively.

Materials and Methods

Chemicals and Reagents

The reagents used during the experimental studies are all of analytical purity and were purchased from Merck and Sigma companies. All of the solutions used were prepared with ultra-pure water with 18.2 MΩ.cm resistance obtained from MP Minipure Dest Up device.

Venlafaxine was purchased from Sigma Aldrich (St. Louis, MO, USA). The standard stock solutions of venlafaxine were prepared in methanol by using brown flask. The standard solutions stocked in a dry place and protected from the light. The standard model solution was newly prepared after diluting of the main solutions with methanol. SPE frits and SPE cartridges were purchased from Agilent Technologies (California, USA).

Instrumentation

In all chromatographic measurements, Shimadzu (Prominence) HPLC (Kyoto, Japan) device was used. HPLC device used; It has equipped with LC 20 AD quaternary pump, SPD-M20 A DAD detector, DGU-20A vacuum degasser and CTO-10 AS VP column furnace equipment. Direct determination parameters for optimized with HPLC for venlafaxine active substance before enrichment experiments. Preliminary trials with different columns were performed as this fixed phase and good peak separation was obtained with the Phonomex Luna® Phenyl-Hexyl column. Many trials have been made in isocratic and gradient elution modes with aqueous solutions of organic characterizing phases such as methyl alcohol, ethyl alcohol and acetonitrile, containing buffers of different pH. Experiments continued until significant peaks for venlafaxine were obtained. A summarization of HPLC conditions after optimization was shown in Table 1. As can be seen in Figure 1, the peak of the venlafaxine molecule was observed clearly under the optimized conditions. Peak height and area increase with concentration as expected.

Table 1. HPLC Conditions for Venlafaxine Determinations

Parameter	HPLC Conditions
HPLC Mode	Isocratic elution
Mobil Phase	%10 Methyl alcohol % 60 pH 3 (0.02M) Phosphate Buffer %30 Acetonitrile
Flow Rate	1 mL/min
Execution time	20 dk
Colon	Luna® 15 µm Phenyl-Hexyl 100 Å, 250× 50 mm, AXIA™ Packed, Ea
Colon temperature	40 °C
Injection volume	10 µL

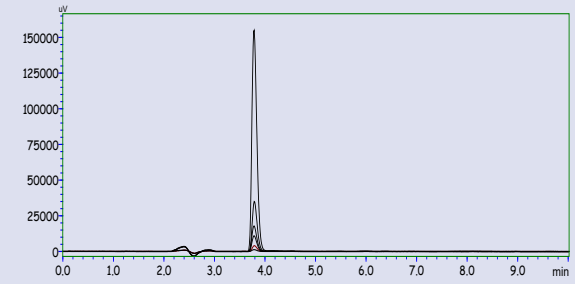

Figure 1. The obtained chromatogram for venlafaxine molecule

Fabrication and Characterization Fabric Phase Support Material

Among all tested membranes, sol-gel carbowax (CW-20 M) sorbent, coated on cellulose FPSE media, was the most efficient.

FPSE membranes were synthesized in the International Forensic Research Institute laboratory (Department of Chemistry and Biochemistry, Florida International University, Miami, FL, USA) according to the procedure described in our previous studies [15,16].

Briefly, a 100 cm^2 segment of cellulose fabric was first soaked with deionized water for 15 min under constant sonication. The fabric was then cleaned with deionized water, followed by soaking in 1 M NaOH solution for 1 h under sonication. The base treated fabric was then washed several times with ample deionized water, followed by treating with 0.1 M HCl solution for 1 h under sonication. Then, the material was washed with a deionized water and finally dried in an inert atmosphere overnight. The dried fabric was stored in a clean glass container until coating process. For modification of fabric phase surface, the sol–gel short-chain poly (ethylene glycol) sorbent was prepared by using a facile sol–gel synthesis approach developed in-house [17,18]. The sol solutions were prepared by sequential mixing of organic polymer or modified silane precursor/methyl trimethoxysilane (MTMS) (acting as sol-gel precursor)/acetone/CH_2Cl_2/TFA/water at various molar ratios as explained in these references. The solution was thoroughly mixed by vortexing for 3 min, centrifuged for 5 min, and sonicated for 2 min. Following centrifugation, the clear supernatant part of the sol solution was transferred to a clean, amber coloured reaction glass

bottle. The cleaned and pre-treated cellulose fabric substrate was then immersed into the sol solution, for a predetermined amount of time, to creating the sol–gel coating. The FPSE media was then cut into 1.5 cm pieces for future use and stored in air-tight containers to prevent contamination.

Characterization of fabric phase material was carried out by using FTIR, SEM technical in our previous studies. The detailed information can be found in these published articles [19–21].

FPSE Procedure

Separation and pre-concentration process based on FPSE were performed by optimizing parameters such as pH, desorption solvent selection and amount, adsorption time, vortexing time. This step permits to eliminate materials impurities and to activate functional groups for subsequently interactions. The membranes were then rinsed in ultra-pure water to remove organic solvent residues before the insertion into the sample for the extraction process.

1.5-cm² SOL-GEL CW20M fabric phases are added to 50 mL falcon tubes before they are passed through distilled water / ACN-MeOH solution / distilled water and washed. Then 20 mL of distilled water on them respectively; 2 mL of pH: 10 Br buffer 1 mL venlafaxine model solution is added and its volume is completed to 50 mL with distilled water. The covers of the falcon tubes are well closed and placed in the shaker device and left to shake at 100 rpm for 60 minutes. After shaking is completed, the tubes are taken and the fabric phase is separated from the liquid phase and 800 µL of ethyl alcohol solution is added to them and vortexed for 60 s. That is, the transition of analyte components attached to the fabric phase to solvent ethyl alcohol is provided. This solvent liquid phase samples are taken into the injectors and filtered through a 0.45 µm PTFE membrane filter and transferred to the vials. It is given to the HPLC device.

Preparation of Synthetic and Normal Urine Solution

As the application area of the developed method, two different samples were carried out including, synthetically and normal urine samples which were just used as spiked samples.

Normal urine sample was taken from a healthy volunteer was directly subjected to the developed method. The human urine samples were collected in a capped test tube from a healthy volunteer free from any kind of medication who had been informed about the perimental procedure and the nature of the study.

Synthetic urine solutions were prepared to be used in the literature to represent urine, which is one of the sample groups where antidepressant agents are most frequently monitored, and was used during the application [11]. Synthetic urine solution was prepared as explained [11]: 6.25 g urea, 0.27 g $CaCl_2.2H_2O$, 0.25 g NH_3Cl, 0.4 g KCl, 0.35 g Na_2SO_4, 0.35 g KH_2PO_4, 0.73 g NaCl were weighed and dissolved in some distilled water and its volume was completed to 250 mL in volumetric flask. The pH was then adjusted to pH6 with 0.1 M HCl solution. It was transferred to an amber coloured bottle and stored at + 4˚C.

Results and Discussion

Extraction Optimization Experiments

The objective is to keep the analyte type in the fabric phase at the highest possible level and to separate it from other substances in the environment, and after the separation process is achieved, all of the analytes in the solid phase pass into the solvent. Preliminary trials were made to determine the necessary parameters to achieve this. It was aimed to obtain a fast and easy separation process and to obtain the highest concentration of analyte by using as little amount of organic solvent as possible. Accordingly, a chromatographic method before FPSE experiments was developed for Venlafaxine molecule by optimizing all parameters.

pH Effect

The pH of the medium is a very important parameter for it affects the interaction of the analyte with the fabric phase and the reactions between the species. 2 mL range of pH 2-10 Britton Robinson (BR) buffer was added to all tubes including 200 ng mL^{-1} venlafaxine, and their volume was completed to 50 mL by ultrapure water. After FPSE method was applied, the content of venlafaxine was determined by HPLC-DAD system. The obtained results were shown in Figure 2. It is seen that the optimum pH value in the enrichment processes is pH 10. As a result, it has been understood that pKa value of venlafaxine in the alkaline region. It becomes positively charged in the alkaline region. We investigated from the literature that venlafaxine has a pKa value of 9.40[22], which shows suitability with our study.

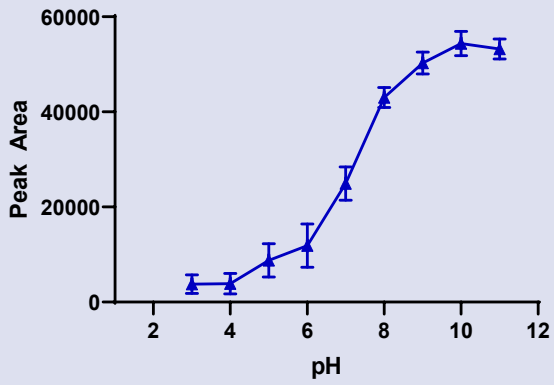

Figure 2. Suitable pH for the proposed method

Eluent Type and Volume

Selecting of eluent for desorption process is an important factor that a solvent can completely desorbed analyte molecule from solid phase surface without damaging the HPLC device. The target molecules are separated from the liquid phase and cling to the phase

after shaking. The solvent chosen for this purpose should be in accordance with the executive phase of the HPLC system. For this purpose, a solvent series were used containing methanol, ACN, isopropyl alcohol (IPA), ethanol, water and acetone. The results of this study were showed in Figure 3. It is seen that the most suitable solvent for desorption in FPSE was determined as ethanol. This result shows that our active substance can be taken better in solvents with high organic character.

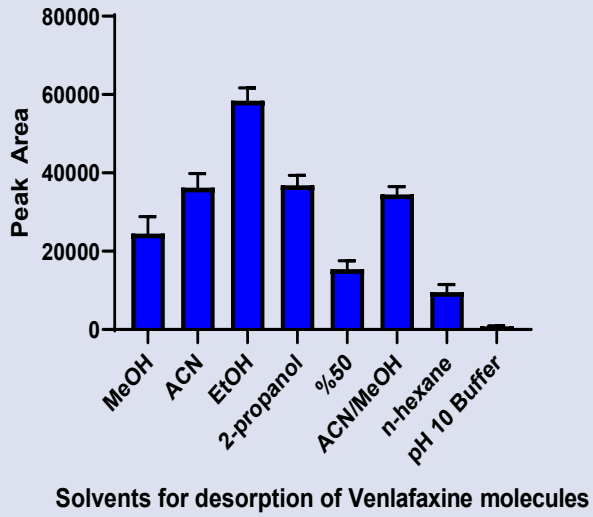

Figure 3. Optimization of desorption solvent

The amount of solvent must be within the range that can be analysed in the HPLC instrument. Volume of desorption solvent was studied by adding ethanol in the range of 200-1500 µL. At the end of these processes, the analyte components attached to the fabric phase were transferred to ethanol phase and this solvent phase were taken with an injector filtered through a 0.45 µm PTFE membrane filter and put into vials to submit to the HPLC device. As can be seen in Figure 4, the highest signals were obtained with 800 µL of ethanol and this volume was selected for desorption process.

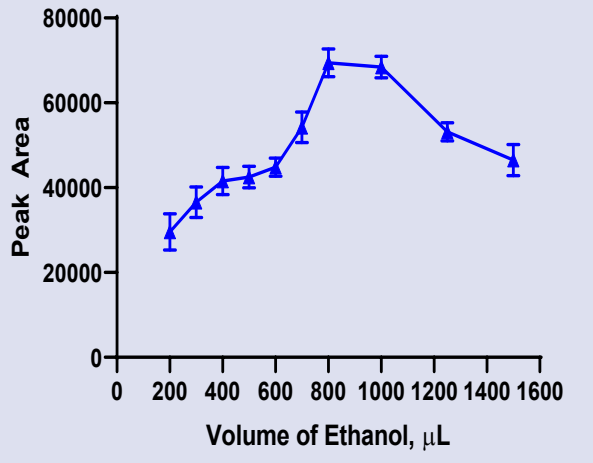

Figure 4. Suitable volume of ethanol for proposed method

Adsorption Time

The adsorption time, also known as the contact time required for the transition of analyte types from the environment where the analyte is located, is an important parameter. The tubes were shaken in shaker at 1000 rpm for 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 minutes, respectively. The results obtained are shown in Figure 5. It is seen that the optimum adsorption time in enrichment processes to be made after that is 60 minutes. It keeps the maximum amount it can hold after 60 minutes. If the tubes are left in more shakers, the adhering substances start releasing, because the interaction is physical, so the signal starts to decrease as the time increases.

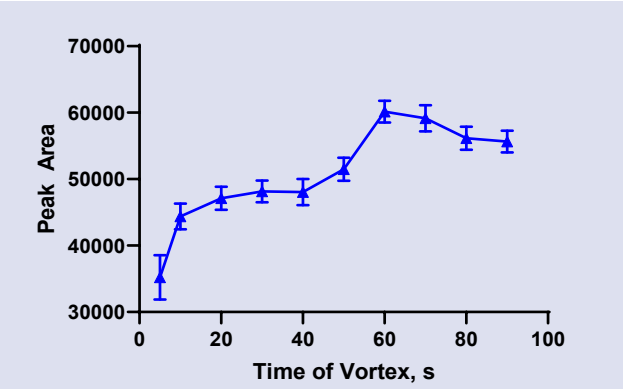

Figure 5. Suitable vortex time for the proposed method

Analytical Performance of the Developed Method

After determining the most suitable experimental conditions for fabric phase extraction, model solutions containing venlafaxine in different concentrations were prepared and each developed FPSE method was applied to determine the linear working range. With the help of analytical signals monitored with the DAD detector, the linear working range for venlafaxine molecule was determined to be 15-750 ng mL^{-1}. All analytical parameters of the developed method are collectively presented in Table 2.

Table 2. Analytical merits of the proposed method

Parameter	Before FPSE	After FPSE
Linear dynamic range	1.00-20.00 µg mL^{-1}	15.00-750.00 ng mL^{-1}
Limit of detection, LOD	0.32 µg mL^{-1}	4.28 ng mL^{-1}
Limit of quantification, LOQ	0.96 µg mL^{-1}	14.14 ng mL^{-1}
RSD(%) (for 300 ng mL^{-1})	3.10	2.40
Calibration sensitivity	11.42	822.24
Correlation Coefficient (R^2)	0.9972	0.9972
Pre-Concentration Factor[a]	-	62.50
Enhancement Factor[b]	-	72.00

[a] Pre-concentration factors (PF) were calculated by using the ratio of the initial solution volume (50 mL) to the last elution solvent volume (0.8 mL).

[b] The enhancement factors (EF) were found from the ratio of the slope of calibration curve of the analytes after MSPE application to that of prior FPSE application.

Analysis of Urine Samples

Simulated urine and normal urine samples were analysed in order to investigate the applicability of the proposed method by means of recovery tests. Venlafaxine concentration of the studied samples were analysed by using the optimized method and results were shown in Table 3. A known concentration of Venlafaxine was spiked to samples because none of samples was contained it as expected. The recoveries of target molecule in the spiked samples were in the range of 97.5-104.2 %. These satisfactory results demonstrate that the developed FPSE based HPLC-DAD method is suitable for trace determination of venlafaxine molecule in the urine samples.

Table 3. Analysis of urine samples

Sample	Added ng mL^{-1}	Found[a,b] ng mL^{-1}	%RSD	% Recovery
Synthetic urine solution	-	<LOD	-	-
	100.0	97.5 ±5.4	5.5	97.5
	200.0	195.1±9.4	4.8	97.8
Normal urine sample	-	<LOD	-	-
	100.0	103.7± 3.9	3.8	103.7
	200.0	208.4±9.8	4.7	104.2

[a]Mean value ± standard deviation found for three replicate measurements at 95% confidence level

[b]Concentrations in a 50 mL solution obtained after sample preparation

Conclusions

A new, sensitive, simple and reliable HPLC based method was developed for the determination of Venlafaxine. The present method offers a simple extraction procedure. The proposed FPSE-HPLC-DAD procedure allows the reliable analysis of Venlafaxine which have not been determined in previously reported analytical method. When we consider it from this perspective even also, it can be concluded that the study is original. It offers a different perspective to the literature in terms of contributing to future studies. The developed method has the advantage of being fast and easy. Analysis of drug active ingredients, especially in complex biological environments, is a very difficult task. For this purpose, mostly complex analysis setups and expensive devices are used. In order to perform these analyses with basic laboratory equipment and a classical HPLC system that can be found in every laboratory, fabric phase extraction was applied as a pre-treatment and the analytical validation of the FPSE-HPLC-DAD-based method developed with trace levels of venlafaxine in model solutions was performed and all parameters were given in Table 2.

In this study, a method for chromatographic determination of venlafaxine molecule after enrichment was applied for the first time. The method increases the low concentrations of the active ingredient of venlafaxine to levels that can be determined by conventional HPLC systems with a simple pre-application system that can be found in every laboratory.

Acknowledgment

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Conflicts of interest

All authors declare that they have no conflict of interest.

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