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Detection and determination of venlafaxine in liver tissue by colour tests, TLC, UV-spectroscopy, HPLC with multi-wave detection

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ABSTRACT

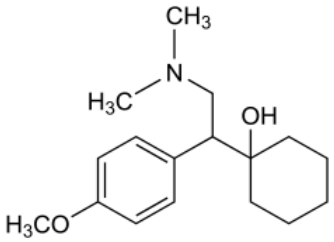
Venlafaxine is most commonly prescribed for major depression and/or anxiety disorders. Fatal Venlafaxine overdoses have been reported. Forensic identification of an analyte requires the use of at least two methods that employ different physical and chemical principles. In the present work, Colour Tests, TLC, UV-spectroscopy, HPLC with multi-wave detection have been used for detection and determination of Venlafaxine in the liver tissue. The effective method of Venlafaxine isolation from the biological sample by drug elution with chloroform from the tissue homogenized by its grinding with anhydrous sodium sulphate followed by the extraction clean-up procedure with the n-hexane-acetonitrile solvent system has been developed. Resolution of the method was 51.3 % (RSD=5.5 %). Quantitative determination was performed by HPLC at 280 nm over the concentration range of 23.6–400 μg/mL with accuracy of 100.0–102.7 % and precision of 0.2–11.2 % depending on the concentration level. The calibration curve was represented by the following regression $Y=1.64\cdot10^{-4}X$, LOQ=23.6 μg/mL ($10SD_a^2/b$), LOD=15 μg/mL (S/N=3:1) for Venlafaxine in methanol solutions. It has been shown that the matrix co-eluting components do not interfere with the quantitative determination of Venlafaxine by HPLC at wavelength of 280 nm.

Keywords: Venlafaxine, liver tissue, Colour Tests, TLC, UV-spectroscopy, HPLC with multi-wave detection.

IINTRODUCTION

Depression and anxiety are the most frequent psychiatric disorders commonly found [1, 2]. Pharmacotherapy involves the prescription of a wide range of novel antidepressants such as selective serotonin reuptake inhibitors (SSRIs) and selective noradrenaline and serotonin reuptake inhibitors (SNRIs) [3, 4].

Venlafaxine (brand name is Effexor or Efexor) (1-[2-(Dimethylamino)-1-(4–methoxyphenyl)ethyl]cyclohexanol hydrochloride) is an antidepressant in the group of SNRIs. Venlafaxine also inhibits, to a lesser extent, dopamine reuptake [5, 6].



The compound exists as racemate with both active R(+) and R(–) enantiomers and has the empirical formula of $C_{17}H_{27}NO_2\cdot HCl$. Its molecular weight is 313.87; the melting point is from 102° to 104°; it is soluble in water (572 g/L), dilute hydrochloric acid, ethanol and chloroform. The partition coefficient (log P(octanol/water)) is 0.43; the

volume of distribution (V_d) is 4–12 L/kg; protein binding in plasma for Venlafaxine and O-desmethylvenlafaxine is 30% [7, 8].

Venlafaxine is used for the treatment of major depressive disorders, generalized anxiety disorder and panic disorder [9]. In 2007 Venlafaxine was the sixth most commonly prescribed antidepressant of the US retail market, with 17,2 million prescriptions [10].

Nausea, somnolence, dry mouth, dizziness, insomnia, constipation, nervousness are its common side effects [8]. But the most serious complications are neonatal withdrawal symptoms [11], sexual dysfunction, increased suicidal risk [12–15].

Several Venlafaxine fatal intoxications have been reported [7, 8, 16, 17–20]. 30 g overdose is associated with significant risk of death. In fatal cases drug concentrations were in the range of 41–89 mg/L for the blood and in the range of 21–430 mg/kg for the liver, the value for the brain was 543 mg/L, the average value for the kidneys was 420 mg/kg, in the urine the average value was 125 mg/kg and it was 11 mg/L in the gastro-intestinal tract [7]. Postmortem Venlafaxine redistribution occurred in the tissues [21].

A lot of methods of Venlafaxine determination (with or without its metabolites) in plasma and the whole blood are developed using the liquid chromatography (HPLC) with different kinds of detection. UV [20, 22–26], DAD [27, 28], fluorescence [29–31], and mass spectrometer [32–36] were used as detectors. The gas chromatographic method (GC) with NPD [20, 37] and MSD [38–42] were applied. Electrokinetic capillary chromatography was described for Venlafaxine [43–46]. Liquid-liquid extraction (LLE) [23, 25–29] and Solid phase extraction (SPE) [22, 32, 33, 40, 41], SPE micro extraction [39] were used as sample preparation methods.

Venlafaxine determination in brain tissue was performed by GC-MS method [41]. The sample preparation consists of elution of the drug by acetonitrile from the homogenized tissue followed by the SPE clean-up procedure. The recovery reported was 67 % (6 % RSD)–90 % (18 % RSD).

The methods adduced above have been developed mostly for the TDM [22, 27, 28, 40], bioequivalence [35], pharmacokinetic studies [36] of Venlafaxine.

While human fluids are the main samples in the bioanalytical research, the human tissues are valuable samples in the postmortem toxicology. The liver is the most important specimen for such kind of the analysis because of the great amount of the tissue available, higher concentrations of many basic drugs in the liver comparatively to blood, relatively stable concentrations in the liver for those drugs, which undergo postmortem redistribution [47]; the database of liver drug postmortem concentrations available in the literature is also relatively large compared to the relevant data for other tissues. The main disadvantage of the liver as a specimen is that it is fatty and the sample preparation procedure must include some additional clean-up steps to minimise the matrix effect of the tissue [48].

Extraction of admixtures with a suitable solvent under certain conditions (back extraction method) and SPE are the most widely used for the extract purification [48, 49]. SPE is affective to remove interfering compounds and to concentrate analytes, gives good recovery and reproducible results. Disadvantages are the cost of the SPE material and the time-consuming optimization procedure, including the selection of a suitable sorbent material, sample loading, column conditioning, washing and solvents for the analyte elution [41]. Thus it should be the useful instrument for rapid and efficient processing of multiple samples. Back extraction method, especially in combination with TLC-purification, is still frequently used in analytical toxicology, especially for purposes of the screening analysis of non-serial samples [38, 48–50]. In addition, development of a LLE procedure is less time-consuming.

The legal aspect of Forensic Toxicology generates a set of principles to be followed in the analysis. The main principle establishes that forensic identification of an analyte requires the use of two methods that employ different physical and chemical principles (SOFT/AAFS Guidelines Committee 2006) [51]. GC-MS and especially HPLC-MS methods are ideal instruments for this purpose because each of them is a combination of two very different analytical methods: chromatography and spectroscopy. But such methods as Colour Tests, Thin Layer Chromatography (TLC) remain popular for many reasons. They are simple to perform and no extensive training is required. As such, they are the methods of appeal in situations where laboratory facilities may be very limited [48, 49].

The aim of this work was to develop efficient method of Venlafaxine isolation from the liver tissue followed by the extract analysis with Colour Tests, TLC, UV-spectroscopy, HPLC with multiwave detection.

EXPERIMENTAL SECTION

2.1. Reagents

Venlaxor (75 mg) tablets containing Venlafaxine hydrochloride were purchased from Grindex (Riga, Latvia).

Methanol and water were of HPLC-grade (Merck, Darmstadt, Germany), acetonitrile was of HPLC-grade (Sigma-Aldrich Laborchemikallen, GmbH), platinic chloride (99.995 % trace metals basis) was obtained from Sigma-Aldrich (USA), sodium sulphate and potassium iodide were analytical grade (Chimmed Company, Moscow, Russia).

All other chemicals were of analytical grade or better and were purchased from Sigma-Aldrich, (USA): perchloric acid (70 %), lithium perchlorate (trihydrate), sulphuric acid (95–98 %), sodium nitrite, ammonium vanadate, ammonia-solution 25 %, hydrochloric acid (37 %), chloroform, *n*-hexane, ethyl acetate, *n*-butanol.

Acidified iodoplatinate solution was prepared by dissolving 0.25 g of platinic chloride and 5 g of potassium iodide in bidistilled water to produce 100 mL, followed by adding 5 mL of hydrochloric acid to 100 mL of iodoplatinate solution obtained.

Mandelin's reagent was prepared by dissolving 0.5 g of ammonium vanadate in 1.5 mL of distilled water and diluting to 100 mL with sulphuric acid followed by filtration of the solution obtained through glass wool (the reagent must be freshly prepared).

Liebermann's reagent was obtained by dissolving 1g of sodium nitrite in 10 ml of concentrated sulphuric acid while grindig in a mortar (the reagent must be freshly prepared).

Eluent A for HPLC (0.2 M lithium perchlorate-0.005 M perchloric acid) was prepared using *Solution 1* and *Solution 2*. *Solution 1* (4.1 M aqueous solution of lithium perchlorate) was obtained by dissolving 330 g of $LiClO_4 \cdot 3\ H_2O$ in 450 ml of bidistilled water while stirring and heating to 50 °C, the solution obtained was cooled to room temperature and the volume was diluted to 500 mL with bidistilled water. The solution was filtered through a 0.45 µm pore size membrane filter. *Solution 2* (4 M aqueous solution of $LiClO_4$ in 0.1 M $HClO_4$) was prepared by diluting 2.2 mL of perchloric acid to the volume of 250 mL with *Solution 1*. *Eluent A* was prepared by diluting 10 mL of *Solution 2* to 200 mL with bidistilled water.

The drug-free post-mortem liver tissue was obtained from the Forensic Toxicological Department of the Regional Office of Forensic Medical Examination (Kharkov, Ukraine).

2.2. Extraction of Venlafaxine from Commercial Tablets

Venlafaxine hydrochloride was extracted from commercially available tablets. Thirty tablets of the drug were weighted, crushed to powder and the powdered drug was extracted by chloroform (60 mL) with heating at 30–40 °C. Chloroform was separated by filtration through a paper filter. The residue was extracted again with 20 mL of chloroform followed by filtration. All chloroform fractions were combined together and the organic solvent was evaporated on a water bath at 40 °C. The residue was weighed. The purity of the drug extracted was ascertained by recording its melting point, TLC, UV spectra.

2.3. Stock solution

A stock solution of Venlafaxine (1.00 mg/mL) was prepared by dissolving 25.00 mg of the drug in 25 mL of methanol. Working standard solutions for the calibration curve were prepared by adding measured volumes of the stock solution to 10 mL of methanol. Ten working standard solutions in the concentration range of 10–400 µg/mL were prepared.

2.4. Instrumentation

High Pressure Liquid Chromatograph "MiLiChrome A-02" (EcoNova, Novosibirsk, Russia), consisting of Double Syringe Gradient pumping system, autosampler, column oven, multiwave UV-detector. Data acquisition and integration were performed by means of "Multichrom" software (AMPERSAND LTD, Moscow, Russia). An "Analytik Jena AG" UV/Vis-spectrophotometer (Kundendienst, Germany) (data were processed using WinASPECT software, version 2.3.1.0). A pH-meter 5123 (Elvro, Wroclaw, Poland). A water-bath LW-4 (Bytom, Poland). Volumetric flasks – 10 mL, 25 mL, volumetric pipettes, Class A, glass – 0.2; 1; 2; 5 mL, a burette with a tap – 25.0 mL, glass vials were purchased from Simax (Czech Republic). Membrane filters, type HA (with the pore size of 0.45 µm) were purchased from "Millipore Corporation" (USA). A whatman quantitative filter paper, ashless,

grade 589/2, white ribbon, circles 110 mm in diameter (Sigma-Aldrich). Glass capillaries were calibrated with the help of a micropipette (0.200 mL).

2.5. Sample preparation

Method of Venlafaxine isolation from the liver tissue. The liver tissue (5 g) shredded into 1–2 mm size pieces was spiked with the aqueous solution of Venlafaxine containing 500 μg of the antidepressant and left standing for a day. Then the tissue was homogenized by trituration with a triple amount of anhydrous sodium sulphate. The resulting granular mass was placed into a glass burette and Venlafaxine was eluted with 100 ml of chloroform dropwise using a separating funnel. The eluate obtained was washed with water alkalified by 10 % ammonia-solution (pH 8.0) twice, evaporated on a water bath at 40 ºC and the dry residue was dissolved in 10 mL of *n*-hexane. Then Venlafaxine was extracted four times with 10 mL portions of acetonitrile. This was followed by acetonitrile solution evaporation at 40 ºC and reconstitution in 25 mL of chloroform using the appropriate volumetric flask.

2.6. Colour Tests

The thin-layer chromatography method of the sample spotting was used in performing the Colour Tests. Some 1 mL aliquots of the drug-containing chloroform extract concentrated to the minimum volume (~ 0.05 mL) were applied on the pieces of chromatography plates with the help of capillary, the solvent was avaporated and the spots were treated with the following reagents: Mandelin's reagent and Liebermann's reagent. Standard methanol solution of Venlafaxine (with the volume of 10 μL, 1000 μg/mL) and blank (drug-free) chloroform extract (1 mL) were tested at the same time as a test sample.

2.7. Thin Layer Chromatography

The plates were activated by heating in an oven at 100 °C. 0.5–1.0 mL aliquots of the final chloroform extracts obtained from the tissue spiked with Venlafaxine and from the blank (drug-free) tissue were concentrated to the minimum volume (~ 0.05 mL) and applied on three TLC plates as spots with the help of a calibrated capillary. 10 μL aliquots of the standard solution of Venlafaxine in methanol (1000 μg/mL) were spotted on these chromatography plates. 5 ml (concentrated to the minimum volume) of the final extract containing Venlafaxine was applied as band only on the chromatography plate, which then was developed in the mobile phase 2 (Table 1). Then the zone in the chromatogram corresponding to this band was not treated by the location reagent. TLC plates were developed in rectangular glass chambers (25 x 25 x 12 cm) using three mobile phases (1, 2, 7 in Table 1). The plates were sprayed with acidified iodoplatinate solution. Venlafaxine was eluted from the chromatogram strip untreated by the location reagent with methanol (the efficient recovery of venlafaxine by elution from the plates was 99.2%), the eluate was evaporated and the residue was reconstituted in 1 mL of methanol.

2.8. UV-spectroscopy

The UV-spectroscopy study was performed over 205–380 nm wavelength range, 10 mm light pathway cuvette was used. The methanol eluate obtained from the chromatography plate was evaporated to the minimum volume and its volume was adjusted to 4 mL with methanol. UV spectrum of the solution obtained was analysed. The reference solution was blank methanol eluate.

2.9. HPLC

The column of 2 mm in id and 75 mm of the total length with C_{18} reverse phase ProntoSIL-120-5-C18 AQ (Bischoff Analysentechnik und Gerate GmbH, Leonberg, Germany) was used. *Eluent A* was 0.2 M lithium perchlorate-0.005 M perchloric acid, *eluent B* – acetonitrile; the gradient elution mode was used: from 5 % *eluent B* to 100 % *eluent B* for 40 min then 100 % *eluent B* for 3 min. The flow rate of the mobile phase was held at 100 μL/min. The oven was set at 40 °C. Detection was performed at 8 wavelengths: 210, 220, 230, 240, 250, 260, 280, 300 nm. The injected volume was 10 μL.

RESULTS AND DISCUSSION

3.1 Detection of Venlafaxine by Colour Tests and TLC

Venlafaxine gives colour with Mandelin's reagent (a green colour, the sensitivity is 4 μg in the sample extract) and Liebermann's reagent (a yellow colour turning to lemon-brown, the sensitivity is 3 μg in the sample extract).

In TLC analysis we tried 7 mobile phases recommended by the Committee for Systematic Toxicological Analysis of The International Association of Forensic Toxicologists (TIAFT) for general TLC-screening of neutral and basic psychotropic drugs. The use of some separation systems (at least 3) with a low Rf value correlation provides the basis for reliable identification of toxic doses of this substances. The Rf values of Venlafaxine are given in Table 1. They are distributed evenly over the full Rf range, and it also complies with the requirements of TIAFT [48]. The mobile phase 2 with the highest Rf value was used for clean-up step by TLC.

The sensitivity of Venlafaxine detection with acidified iodoplatinate solution (violet) was 1 μg of the drug in the spot.

According to the recommendations of TIAFT [48] and UNODC Guidance [52] the Rf values were standardized using reference substances given in Table 2.

Table 1: Mobile phases used in TLC analysis of Venlafaxine

Mobile phase	Rf ± 0.02
1. Chloroform-Methanol (90:10)	0.40
2. Ethyl acetate-Methanol-Ammonia-solution 25 % (85:10:5)	0.81
3. Methanol	0.40
4. Methanol-*n*-Buthanol (60:40)	0.21
5. Methanol-Ammonia-solution 25 % (100:1.5)	0.71
6. Cyclohexane-Toluene-Diethyl amine (75:15:10)	0.63
7. Acetone	0.13

Table 2: Reference substances for mobile phases used in TLC analysis of Venlafaxine

Mobile phase	Reference substances	Rf ± 0.02
1. Chloroform-Methanol (90:10)	Strychnine Procaine	0.33 0.44
2. Ethyl acetate-Methanol-Ammonia-solution 25 % (85:10:5)	Eserine Ambroxol Codeine Tramadol	0.23 0.41 0.49 0.88
3. Methanol	Pachicarpine Strychnine Chlorpromazine Procaine Papaverine	0.08 0.14 0.18 0.75 0.86
4. Methanol-*n*-Buthanol (60:40)	Strychnine Tramadol Diltiazem Piracetam Ambroxol Cinnarizine	0.09 0.30 0.37 0.59 0.81 0.84
5. Methanol-Ammonia-solution 25 % (100:1.5)	Pachicarpine Atropane Halidor Lidocaine Cinnarizine Papaverine	0.09 0.15 0.39 0.77 0.78 0.83
6. Cyclohexane-Toluene-Diethyl amine (75:15:10)	Carbamazepine Strychnine Halidor Bromhexine	0.02 0.18 0.64 0.69
7. Acetone	Halidor Papaverine Lidocaine	0.04 0.38 0.77

The combination of Colour Tests with the TLC data allows to improve the certainty of identification [48]. These methods may be used in the screening analysis of Venlafaxine in the sample extracts.

3.2. Identification of Venlafaxine by UV-spectroscopy

In accordance with the general approach of qualitative application of UV-spectroscopy in the forensic toxicology study an independent method (e.g. chromatography) must prove that the material consists substantially of one absorbing component [48].

Identification of Venlafaxine isolated from the liver was performed after clean-up step by TLC method. The UV-spectrum of the methanol eluate containing Venlafaxine was identical to the spectrum of the standard methanol solution of Venlafaxine (Fig. 1). The wavelengths of the principal peaks and the corresponding specific absorptivity values were: 226±2 (A^1_1=339.0), 277±2 (A^1_1=43.8), 284±2 (A^1_1=37.0).

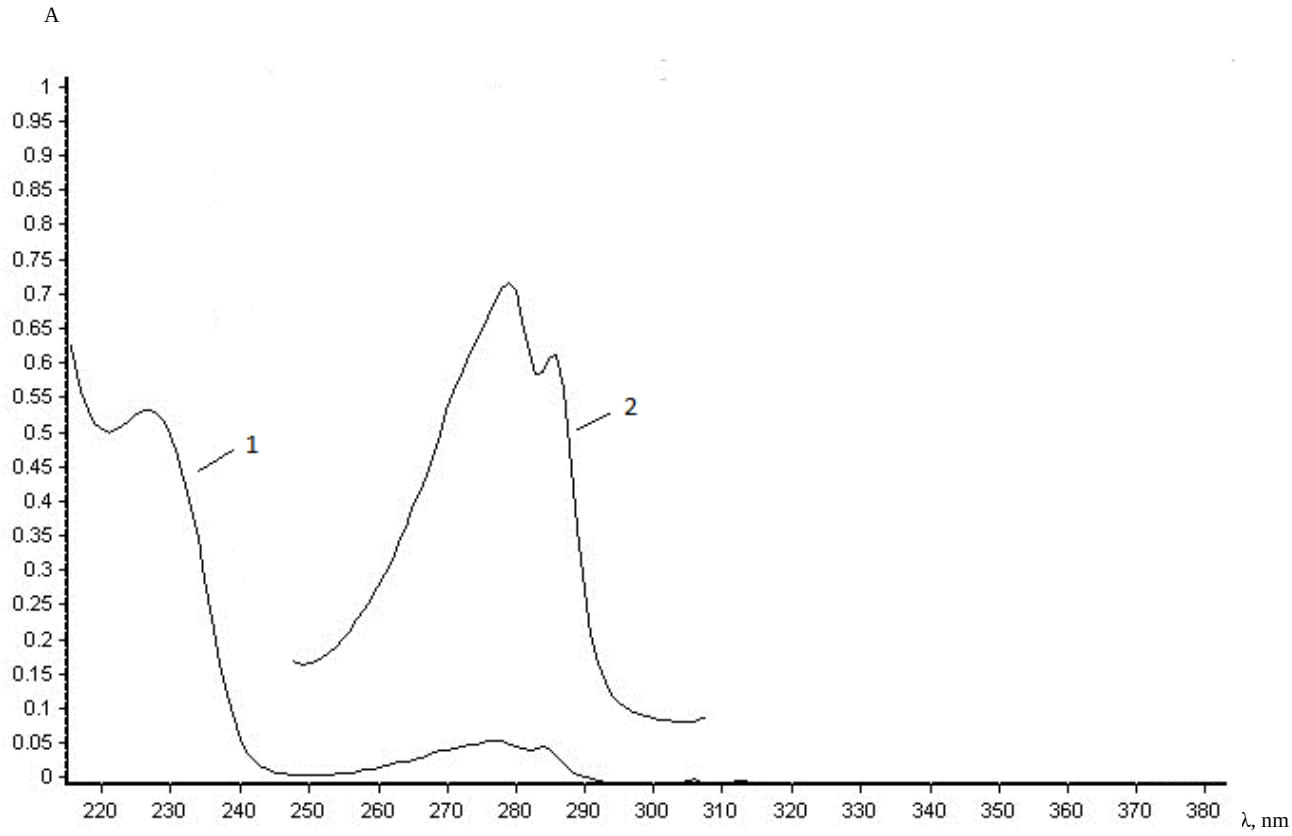


Fig. 1: UV-spectra of Venlafaxine methanol solutions: $5 \cdot 10^{-5}$ mol/L (1), $5 \cdot 10^{-4}$ mol/L (2)

LOD was 3.3 μg/mL regarding the characteristic peak at 277 nm. It was estimated from the intercept standard deviation of the calibration curve obtained by the following equation: LOD=3.3SD_a/b [52] (linearity was in the concentration range of 10.8–300 μg/mL).

The UV-spectroscopy can serve for confirmatory analysis in combination with other analytical data. Further substance identity evidence can be derived from the absorptivity ratios of peaks within a spectrum [48]. We used this approach for identification of Venlafaxine by HPLC with multiwave UV-detection.

3.3. Identification and quantitative determination of Venlafaxine by HPLC

3.3.1. Identification of Venlafaxine

The retention parameters (retention time and retention volume) and the absorbance ratios ($R=S_\lambda/S_{210}$) of Venlafaxine isolated from liver coincided with those in the standard methanol solution (100 μg/mL) (Fig. 2, 3).
Validation was performed by such criteria as precision of retention parameters, limit of detection for Venlafaxine in standard methanol solutions, specificity regarding the biological matrix components.

Suitability of the system and limit of detection

The retention time and retention volume for Venlafaxine were 17.81±0.09 min (RSD=0.20%, ε=0.51%, P=95%, ν=2) and 1781±9 min (RSD=0.20%, ε=0.51%, P=95%, ν=2), respectively; LOD=15 μg/mL by such criterion as S/N=3:1 [51, 52] and LOD=4.0 μg/mL in accordance with the approach of LOD=3.3SD_a/b (SD_a is intercept standard deviation of the calibration curve) for λ=280 nm in methanol solutions. Absorbance ratios ($R=S_\lambda/S_{210}$) are given in Table 3.

Table 3: Absorbance ratios ($R=S_\lambda/S_{210}$) and their presicion (P=95%, ν=2) for Venlafaxine

λ, nm	220	230	240	250	260	280	300
$R=S_\lambda/S_{210}$	1.71	1.88	0.169	0.037	0.100	0.218	0.005
RSD, %	1.70	1.65	0.41	7.75	3.00	1.75	60.0
$\Delta\overline{X}$	0.07	0.08	0.002	0.009	0.007	0.009	0.004
ε%	4.09	4.13	1.18	23.26	7.75	3.95	80.0

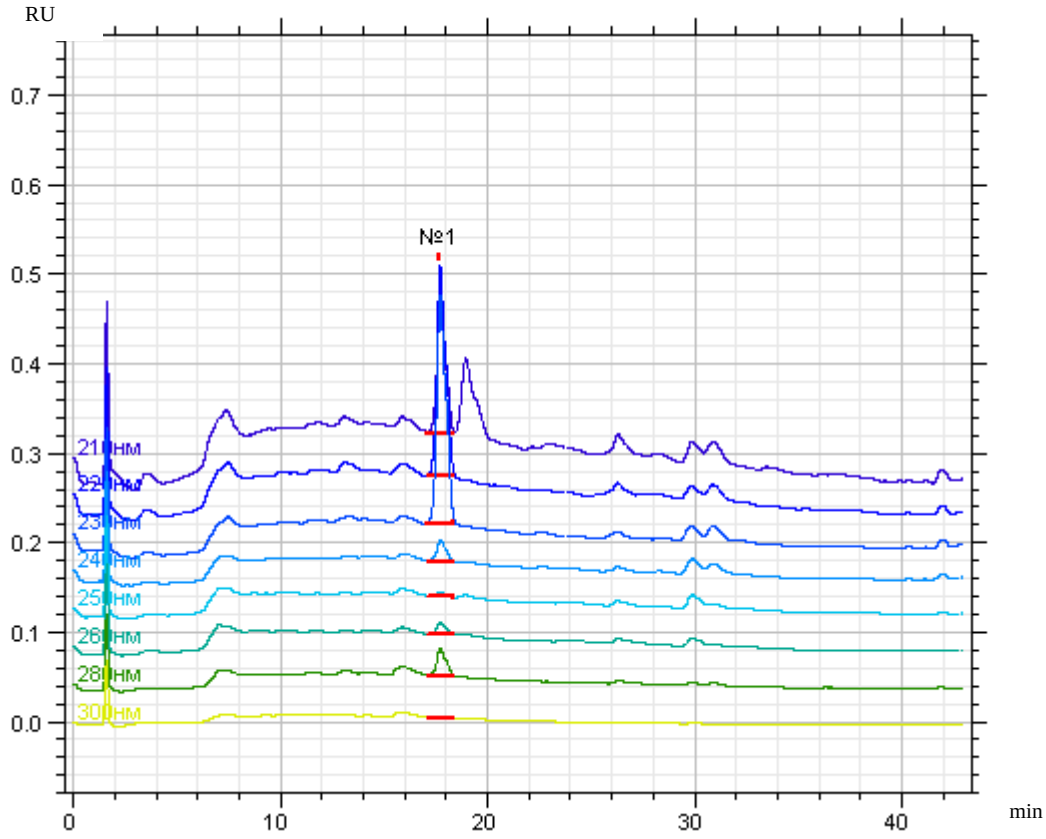


Fig. 2: The chromatogram of Venlafaxine isolated from the liver tissue

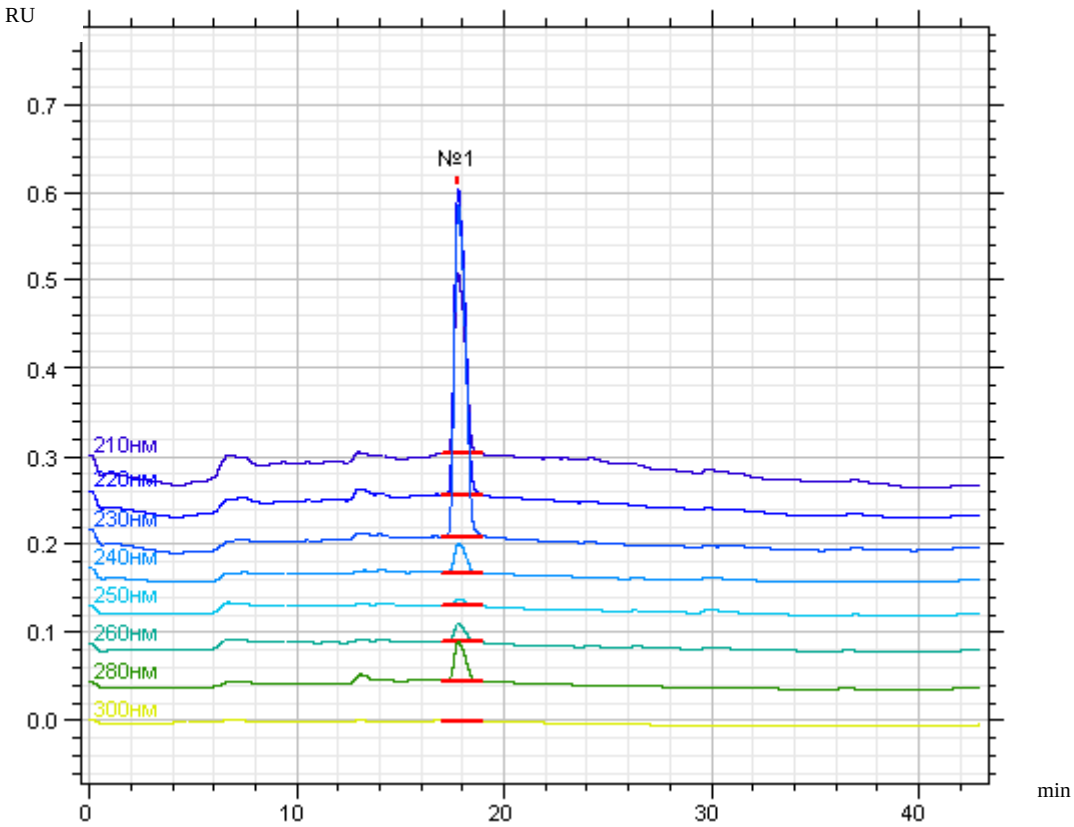


Fig. 3: The chromatogram of the standard solution of Venlafaxine (100 µg/mL)

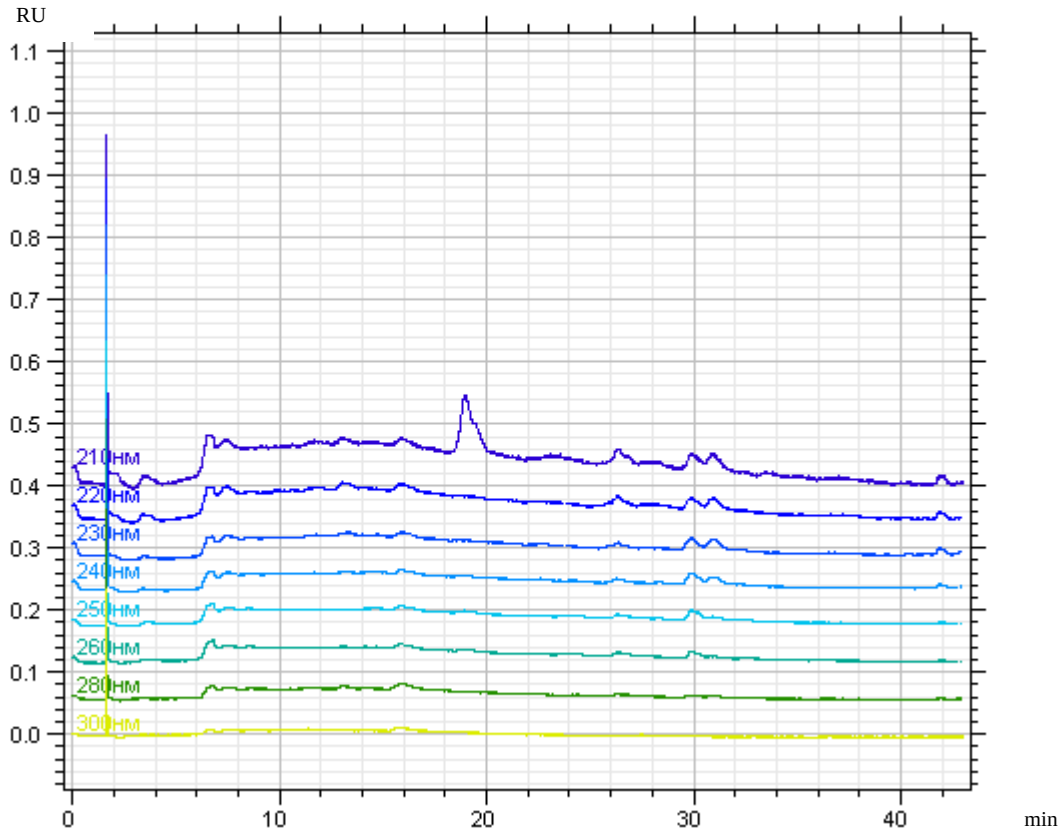


Fig. 4: The chromatogram of the eluate obtained from the blank liver tissue

Specificity

The matrix endogenous components which had the retention time (~19 min) close to that for Venlafaxine (17.81 min) (Fig. 2, 4) were detected only at 210 nm. There is no matrix contribution to absorption of the eluates in the wavelength range from 220 to 300 nm. Peaks of endogenous components and Venlafaxine were separated, so the method of identification developed was specific for Venlafaxine regarding the biological matrix components.

3.3.2. Quantitative determination of Venlafaxine

Quantitative determination was performed at 280 nm. Although the absorption intensity at this wavelength is lower than at 230 nm, but still it is sufficient to determine the lethal concentrations of Venlafaxine in the biological samples. And a significant distance between Venlafaxine absorption peak at 280 nm and the absorbing region of endogenous components eliminates the matrix effect influence on the results of the quantitative analysis. This enabled us to carry out the quantitative determination of Venlafaxine in the eluates using the calibration curve obtained for methanol solutions of the drug.

Validation

The assay linearity, limit of quantification, accuracy and precision at three concentration ranges (low, middle and high) were validated by Venlafaxine methanol solutions. The peak areas obtained were subjected to linear regression analysis.

Linearity and limit of quantification

To evaluate linearity ten working standard solutions in the concentration range of 10–400 μg/mL were measured. Venlafaxine showed linearity in the range of 12.21–400.0 μg/mL and it was represented by the following regression equation: $Y=1.64\cdot10^{-4}X$. We could proceed to the equation of the form $Y=b'X$ as the intercept standard error exceeds its absolute value (Table 4). LOQ=12.21 μg/mL, it was estimated from the intercept standard deviation of the calibration curve by the following equation: LOQ=10 SD_a /b [52]. The results are shown in Table 4.

A***ccuracy and precision***

To determine accuracy and precision five quality control solutions of Venlafaxine in methanol for each (low, middle and high) concentration levels were measured (Table 5). Recoveries of Venlafaxine were 102.7 % at the low concentration level and 100.1–100.3 % at the middle and high concentration levels, indicating a good accuracy of

the method developed. Precision was derived from %RSD values, which were 11.2 % at the low concentration level (must be <20 % [51, 52]) and 0.2–0.3 % at middle and high concentration levels (must be <15–20 % [51, 52]). Therefore the method developed is characterized by the satisfactory precision. Accuracy and precision results are summarized in Table 5.

Table 4: Calibration curve *Y= b'X (Y=bX+a,* the general form), correlation coefficient, LOQ, linearity range for Venlafaxine in HPLC quantitative analysis

Slope *b'* *($SD_{b'}$, $\Delta b'$)*	Intercept *a* *(SD_a, Δa)*	SD_o^2	*r*	LOQ, μg/mL	Linearity range, μg/mL	ethal concentrations, [6]
$1.64 \cdot 10^{-4}$ ($6.14 \cdot 10^{-7}$, $1.4 \cdot 10^{-6}$)	$3.50 \cdot 10^{-4}$ ($2.0 \cdot 10^{-4}$, $4.5 \cdot 10^{-4}$)	$1.5 \cdot 10^{-7}$	0.999	12.21	12.21–400.0	41–89 mg/L (blood) 21–430 mg/kg (liver)

Table 5: Accuracy and precision

Concentration, μg/mL	Amount found (% recovery)	Accuracy, % $\overline{X}$	Precision (%RSD)
15.0	104.5 101.4 103.1 102.4 102.1	102.7	11.2 %
200.0	100.1 100.1 100.6 100.0 100.6	100.3	0.3 %
400.0	100.3 100.2 100.1 99.9 100.0	100.1	0.2 %

3.4. Optimization of the sample preparation method

When optimizing the sample preparation method we tried acetonitrile (as an extragent of Venlafaxine from the ground liver tissue) and chloroform (as the eluent of Venlafaxine from the homogenized liver tissue) followed by the back extraction clean-up step. The aqueous solution-organic solvent extraction system was used: co-eluting matrix components were extracted with diethyl ether from 0.1 M hydrochloric acid Venlafaxine aqueous solution (pH 1), then Venlafaxine was re-extracted with diethyl ether from the alkaline medium (pH 10–11). The TLC-purification was performed in both cases. The recoveries of the methods tested were 43.4 % and 34.5 %, respectively.

Taking into account the Venlafaxine lipophilicity and possible losses of the drug during the back extraction clean-up step described above we carried extraction purification with the *n*-hexane-acetonitrile solvent system. The preliminary model experiments have shown that Venlafaxine was extracted completely from hexane with acetonitrile during four-times extraction. This operation allowed to purify Venlafaxine from fat and lipid co-eluting matrix components.

The method developed has allowed to isolate 51.3 % of Venlafaxine from the liver tissue with satisfactory precision (RSD=5.5 %). The results are shown in Table 6.

Table 6: Recovery and precision of the method developed for Venlafaxine sample preparation using chloroform as an eluent and clean-up step by extraction with the *n*-hexane-acetonitrile solvent system

Amount added to 5 g of the liver, μg	Amount found ($\overline{X}$, % recovery)	SD	% RSD	$SD_{\overline{X}}$	$\Delta\overline{X}$ (P=0.95%, v=4)	ε, %
500	51.3	2.8	5.5	1.3	3.5	6.9

CONCLUSION

The methods of Venlafaxine detection, identification and quantitative determination that are suitable for screening and target analysis of the drug in liver tissue have been developed. Colour Tests, TLC, UV-spectroscopy, HPLC with multi-wave detection have been used. The effective method of the sample preparation considering the Venlafaxine lipophilicity has been proposed. It includes the drug elution with chloroform from the tissue

homogenized by its grinding with anhydrous sodium sulphate followed by the extraction clean-up procedure with the *n*-hexane-acetonitrile solvent system. Quantitative determination has been performed by HPLC. The results of validation have proven that the method developed is specific in relation to the matrix co-eluting components, accurate, precise, sensitive and linear in the range of the expected content of Venlafaxine in the liver tissue in fatal cases.

REFERENCES

[1] CJL Murray; AD Lopez. Global Burden of Disease: A comprehensive assessment of mortality and disability from diseases, injuries, and risk factors in 1990 and projected to 2020, Harvard University Press, Harvard, **1996**; p.p. 5
[2] Nidhi Soni; VK Lal; Shikha Agrawal et al. *J. Chem. Pharm. Res.*, **2013,** 5(3), 7-11.
[3] SM Sampson. *Mayo Clin. Proc.*, **2001,** 76, 739-744.
[4] ND Bateman Antidepressants: Poisonous substances, Elsevier, Amsterdam, **2007;** 587-589.
[5] WA Morton; SC Sonne; MA Verg. *Ann. Pharmacother.*, **1995,** 29, 387-395.
[6] JM Andrews; PT Ninan; CB Nemeroff. *Depression*, **1996**, 4(2), 48-56.
[7] AC Moffat; MD Osselton; B Widdop. Clarke's analysis of drugs and poisons in pharmaceuticals, body fluids and postmortem material, 3th Edition, Pharmaceutical Press, London, **2004**; p.p. 1935.
[8] Randall C. Baselt. Disposition of Toxic Drugs and Chemicals in Man, 9th Edition, Biomedical Publications, Seal Beach,California, **2011;** p.p. 1900.
[9] ME Thase; AR Entsuah; RL Rudolph. *Br J Psychiatry*, **2001**, 178, 234-241.
[10] Top 200 Brand Drugs by Units in 2007. Drug Topics. February 25, **2008**. (Available at: http://drugtopics.modernmedicine.com/drugtopics/data/ articlestandard//drugtopics/072008/491207/article.pdf. Accessed on **2013** November 16).
[11] SC Shivhare; HK Kunjwani; AM Manikrao et al. *J. Chem. Pharm. Res*, **2010,** 2(1), 106-112.
[12] AB Csoka; AS Bahrick; O. Mehtonen. *J. Sex. Med.*, **2008**, 5(1), 227-233.
[13] J Tiihonen; J Lönnqvist; K Wahlbeck et al. *.Arch. Gen. Psychiatry*, **2006**, 63(12), 1358-1367.
[14] A Rubino; N Roskell; P Tennis et al. *BMJ*, **2007**, 334(7587), 242.
[15] DA Brent; GJ Emslie; GN Clarke et al. *Am. J. Psychiatry*, **2009**, 166(4), 418-426.
[16] K. Goeringer; I. McIntyre; O. Drummer. *Forensic Sci. Int.*, **2001,** 121 (1-2), 70-75.
[17] J Mazur; J Doty; A Krygiel. *Pharmacotherapy*, **2003**, 23(12), 1668-1672.
[18] N Banham. *Med. J. Aust.*, **1998**, 169(8), 445-448.
[19] AT Parsons; RM Anthony; JR Meeker. *J. Anal. Toxicol.*, **1996**, 20, 266-268.
[20] C. Long; J Crifasi; D Maginn et al. *J. Anal. Toxicol*., **1997**, 21, 166-169.
[21] KE Rodda; OH Drummer. *Forensic Sci. Int.*, **2006**, 164(2–3), 235-239.
[22] C Frahnert; ML Rao; K Grasmader. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **2003**, 794, 35-47.
[23] G. Tournel; N. Houdret; V. Hedouin et al. *J. Chromatogr. B Biomed. Sci. Appl.*, **2001**, 761, 147-158.
[24] DR Hicks; D Wolaniuk; A Russell et al. *Ther. Drug Monit.*, **1994**, 16, 100-107.
[25] M Matoga; F Pehourcq; K Titier et al. *J. Chromatogr. B Biomed. Sci. Appl.*, **2001**, 760, 213-218.
[26] BB Raut; BL Kolte; AA Deo et al. *J. Liq. Chromatogr. Relat. Technol*. **2003**, 26, 1297-1313.
[27] C Duverneuil; GL de la Grandmaison; P de Mazancourt et al. *Ther. Drug Monit.*, **2003**, 25, 565-573.
[28] K Titier; N Castaing; E Scotto-Gomez et al. *Ther. Drug Monit.*, **2003,** 25, 581-587.
[29] R Waschgler; W Moll; P Konig et al. *Int. J. Clin. Pharmacol. Ther.*, **2004**, 42, 724-728.
[30] R Mandrioli; L Mercolini; R Cesta et al. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci., **2007**, 856, 88-94.
[31] Y H Ardakani; A Foroumadi; MR Rouini. *Daru J. Pharm. Res.*, **2010**, 18(2), 97-102.
[32]. Bhatt J.; Jangid A.; Venkatesh G. et al. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **2005**, 829, 75-81.
[33] J He; ZL Zhou; HD Li. *J. Chromatogr. B Biomed. Sci. Appl.*, **2005,** 820, 33-39.
[34] W Liu; HL Cai; HD Li. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **2007,** 850, 405-411.
[35] BN Patel; N Sharma; M Sanyal et al. *J. Pharm. Biomed. Anal.*, **2008**, 47, 603-611.
[36] F Qin; N Li; T Qin et al. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.*, **2010**, 878, 689-694.
[37] MA Martinez; CS de la Torre; E Almarza. *J. Anal. Toxicol.*, **2002**; 26(5), 296-302.
[38] S Paterson; R Cordero; S Burlinson. *J. Chromatogr. B.*, **2004**, 813(1–2), 323-330.
[39] C Salgado-Petinal; JP Lamas; C Garcia-Jares et al. *Anal. Bioanal. Chem.*, **2005**, 382, 1351-1359.
[40] SMR Wille; KE Maudens; CH Van Peteghem et al. *J. Chromatogr. A*, **2005**, 1098, 19-29.
[41] SMR Wille. Quantitative analysis of new generation antidepressants using gas chromatography-mass spectrometry. Applications in clinical and forensic toxicology, *Thesis submitted to obtain the degree of Doctor in Pharmaceutical Sciences*, Ghent University, **2008**; p.p. 332 (Available at: http://www.zna.be/nl-BE/overZNA/Medisch%20aanbod/Klinische%20Biologie/~/media/Files/Medische%20Diensten/Z21%20Klinische %20Biologie/LaboExterneWebsite/Review%20antidepressiva.pdf.ashx. Accessed on **2013** November 16).

[42] I Papoutsis; A Khraiwesh; P Nikolaou et al. *J. Pharm. Biomed. Anal.*, **2012**, 70, 557-562.
[43] L Labat; M Deveaux; P. Dallet et al. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*., **2002**, 773, 17-23.
[44] S Rudaz; E Calleri; L Geiser et al. *Electrophoresis*, **2003**, 24, 2633-2641.
[45] S Fanali; S Rudaz; JL Veuthey et al. *J. Chromatogr. A*, **2001**, 919, 195-203.
[46] NC Purdeli; D Balalau1; M Ilie1 et al. *Farmacia*, **2010**, 58(1), 62-69.
[47] O H Drummer. *Forensic Sci. Int.***, 2004**, 142(2–3), 101-113.
[48] S Jickells; A Negrusz. Clarke's Analytical Forensic Toxicology, Pharmaceutical Press, London, **2008**; p.p. 648.
[49] RJ Flanagan; RA Braithwaite; SS Brown et al. Basic Analytical Toxicology, World Heath Organization, Geneva, 1995.
(Available at: http://www.who.int/ipcs/publications/training_poisons/basic_analytical_tox/en/index.html. Accessed on 2013 November 16).
[50] A. Imran; H Afzal; S Kishwar et al. *J. Appl. Biopharm. Pharmacokinetics*, **2013**, 1, 12-17.
[51] SOFT / AAFS Forensic Laboratory Guidelines, **2006**; p.p. 1-24 (Available at: http://www.soft-tox.org/files/Guidelines_2006_Final.pdf. Accessed on **2013** November 16).
[52] Guidance for the Validation of Analytical Methodology and Calibration of Equipment used for Testing of Illicit Drugs in Seized Materials and Biological Specimens (United Nations Office on Drugs and Crime, Vienna), New York, **2009**; p.p. 67.