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Determination of venlafaxine and its metabolites in biological materials

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Summary

Venlafaxine (VEN), which was introduced into the therapy in 1990s, is one of the most often used antidepressants. The monitoring of its level in the human organism is recommended, particularly in the case of a patient suffering from other illnesses and treated with different drug substances interfering with VEN. The most popular diagnostic material for the quantitation of VEN is blood. The present study is the review on the methods of determination of VEN and its metabolites in blood and other human diagnostic materials, such as saliva and urine, and also in animal tissues. The paper also sums up the methods of extraction, i.e. liquid-liquid extraction, solid-phase extraction as well as modifications of these methods, such as liquid-phase microextraction and cloud-point extraction. According to the literature, it can be stated that the most popular method for the determination of VEN level and its main metabolite, *O*-demethylvenlafaxine, is HPLC with the UV or spectrofluorimetric detectors. Another separation method used for the determination of VEN is LC with MS. As a stationary phase, the most frequently the C18 column is applied and a mixture of acetonitrile with a phosphate buffer. For the determination of venlafaxine enantiomers, a chiral stationary phase was used.

venlafaxine / *O*-demethylvenlafaxine / HPLC / UHPLC / LC / blood / saliva / urine

INTRODUCTION

Determination of the active substances in biological fluids is important from the pharmacokinetic point of view, in particular during the polypragmasy, when there is a risk of interaction between different drug substances. The interactions can appear at every phase of the pharmacokinetic process. Determination of a drug substance is also necessary in the case of dysfunctions of the liver or kidneys and in the case of active pharmaceutical ingredients with a narrow therapeutic index.

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It is well known that the free fraction of active substances (non-bounded with proteins) is responsible for therapeutic effectiveness of a drug. That is why determination of the level of the active substance is limited to this fraction. A variety of analytical techniques are used for the determination the substances in biological matrices. Most often, the separation methods are applied, i.e. HPLC (*High-Performance Liquid Chromatography*), LC (*Liquid Chromatography)* and GC (*Gas Chromatography*).

There are many antidepressants among drugs determined in biological materials. One of them is venlafaxine (VEN), the second-generation antidepressant drug, introduced into the therapy in 1993. It is prepared as a racemic mixture, but the two enantiomeric forms have different impact on reuptake of neurotransmitters in the synaptic slit. The (–)-(R) enantiomer inhibits both the noradrenaline and serotonin synaptic reuptake,

whereas the (+)-(S) enantiomer inhibits only the serotonin one [1, 2]. From the literature it is also known that VEN acts as a weak inhibitor of the reuptake of dopamine. VEN is mostly used in the treatment of the major depressive disorder and against its recurrences [3, 4]. It is also administered in the therapy of fears, social phobias, sudden fears and agoraphobia.

PHARMACOKINETICS, INTERACTIONS, ADVERSE EFFECTS

The structures of venlafaxine and its metabolites are presented in Fig. 1.

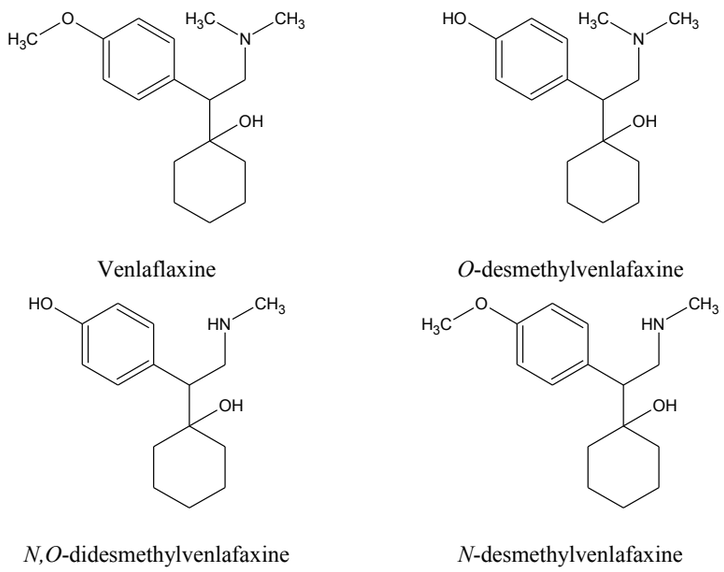

Figure 1. Chemical formulae of venlafaxine and its metabolites

Taking into account its chemical structure, VEN is a derivative of phenylethylamine. From a single dose, VEN is absorbed at least in 92%, and after absorption it goes through the first-pass effect in the liver. Its total biological availability falls in the range of 40 to 45%, depending on the metabolism in an organism [5]. In a blood circulation it is bound by plasma proteins in 27%. VEN is transformed by two enzymes from the group of cytochrome P450, CYP2D6 and CYP3A4, and it is considered as a weak inhibitor of isozyme CYP2D6 responsible for the *O*-demethylation process [6, 7]. Its main and the most important metabolite, *O*-desmethylvenlafaxine (ODV), is produced in the amount of 56%, and has pharmacological activity and effect on reuptake of monoamines, similar to that of VEN. Other metabolites, *N,O*-didesmethylvenlafaxine (16%) and *N*-desmethylvenlafaxine (1%), are biologically inactive [1].

The average half-life of VEN is 5±2 h, whereas that of its main metabolite, ODV, is 11±2 h. The major part of the drug, about 87%, is eliminated by kidneys within 48 h, including the unchanged form (5%), free ODV (29%), coupled ODV (26%), or inactive metabolites (27%). In persons with impaired kidney functions, the half-life in the elimination phase is much longer, which results in the need to reduce the dose taken by a patient [5].

In spite of the fact that VEN and ODV affect only slightly the activity of liver enzymes, they can interact with drugs metabolized by the same isozymes. When the CYP2D6 inhibitors, for instance difenhydramines are applied simultaneously, the VEN level increases in blood [6]. On the other hand, substances inducing this isozyme accelerate VEN metabolism and also reduce the time of the drug activity. Similarly, the use of CYP3A4 inhibitors responsible for ODV metabolism, such as ketoconazole, can increase ODV level in blood, and decrease its clearance. This is especially important in the context of ODV's half-life, because it is longer than that of VEN [6, 7].

In the medical practice, special attention is paid to the fact that a serotonin syndrome can occur when VEN is used in the treatment. This is a life-threatening potential state, especially when the drug is given together with other substances influencing the serotoninergic transmission, *e.g.* monoamine oxidase inhibitors (IMAO) [5]. For this reason these drugs cannot be administered together with VEN. The treatment with VEN should not begin sooner than 14 days after finishing the treatment with IMAOs. A 7-day break is also recommended after ending the treatment with VEN, before starting the administration of MAO inhibitors. The same refers to the treatment with lithium salts.

VEN can enhance the risk of blooding through disordering of thrombocytic activity. It is especially dangerous for patients taking anti-coagulants and inhibitors of thrombocytes. Hence, in these cases VEN must be administrated with particular care. VEN should not impair the functions of brain or inhibit motoric activity, this however depends on personal features of a patient. It can enhance the action of ethanol, and that is why it is not recommended to drink any alcohol during the treatment with VEN, similarly as of other substances which have an impact on the Central Nervous System [5]. Other side effects that can occur after venlafaxine treatment are compiled in Tab.1.

METHODS FOR VEN DETERMINATION IN BIOLOGICAL MATERIAL

The high rank of VEN in depression therapy results in the necessity to develop new analytical methods enabling to monitor its level as well as its metabolites in the organism. These methods should be characterised by such features, which enable quantitation of VEN and its active metabolite over the concentration range of both analytes used in the therapy. The literature data show that therapeutic concentration of VEN ought to be within the range of 0.07 to 0.3 mg/L, whereas that of ODV from 0.2 to 0.5 mg/L. Repeated administration of VEN raises its blood level from 0.07 to 0.27 mg/L, and that of ODV from 0.24 to 0.52 mg/L [8]. Results of pharmacokinetic investigations of VEN have shown that its concentration in blood of 1781 patients ranged between 0.13 and 2.50 mg/L [9]. It was also found that in the group of patients aged 65+ and in women, the VEN level in blood was higher, in spite of the treatment with commonly applied therapeutic doses. In this case it is evident that the level of VEN must be monitored, especially in the group of that category of patients.

The commonly analysed diagnostic material, in which the concentration of VEN and its metabolites was determined, was blood plasma and serum. There were also several attempts to

Table 1. The side effects that can occur owing to venlafaxine treatment [5]

System or organ	Side effect
Blood and lymphatic system	extravasations [c], bleeding from alimentary tract [c] and mucosa e, elongation of the coagulation time [e]
Metabolism and faulty nutrition	increased serum cholesterol [b], weight loss b, abnormal liver function test [e], hepatitis [e]
Nervous system	dry mouth [a], headache [e], decreased libido [b], vertigo [b], nervousness [b], agitation [b], increased anxiety [b], insomnia b, tremor [b], hallucinations [b], myoclonic jerk [c], convulsions [d]
Sense	cycloplegia b, mydriasis [b], vision disturbance [b], dysgeusia [c], ear buzzing [c]
Cardiovascular system	hypertension [b], angiectasia [b], cardiopalmus [b], orthostatic hypotension [c], syncope [c], tachycardia [c]
Respiratory system	increased yawning [b]
Digestive system	nausea [a], loss of appetite [b], constipation b, vomiting [b], diarrhoea [c]
Derma and subcutaneous tissue	diaphoresis [a], rash [c], hair loss [c]
Reproductive and urinary system	malemission [b], impotence [b], dysuria b, pollakiuria [b], dysmenorrhoea [c]
Others	asthenia [b], chills [b], photophobia [c], anaphylaxis [e]

[a] = more common side effects (developed in ≥ 1/10 of treated patients), [b] = common side effects (over the range of ≥ 1/100 to < 1/10 of treated patients), [c] = less common side effects (over the range of ≥ 1/1 000 to < 1/100 of treated patients), [d] = rare side effects (over the range of ≥ 1/10 000 to < 1/1 000 of treated patients), [e] = very rare side effects

use saliva and urine for that purpose. Moreover, VEN was determined in animal tissues, as well, for example in rat liver and brain. For this reason, it was decided to review the literature in order to compile analytical methods used for the determination of VEN and its metabolites in biological materials.

CHROMATOGRAPHIC METHODS

Chromatography is one of the most frequently used separation techniques which enables separation of the sample constituents between two phases. The first one is called the mobile phase, which can be a gas or a liquid and the other is the stationary phase. The separation is based on various interactions between the sample constituents and both phases. The analytes more soluble in the mobile phase are eluted first. For the quantification of VEN and its active metabolites, different chromatographic methods were used, e.g. HPLC (*High-Performance Liquid Chromatography*), LC (*Liquid Chromatography)* and GC (*Gas Chromatography*).

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

HPLC is the most popular LC technique which is characterised by using a high pressure to the chromatographic separation and the samples must always be dissolved in the mobile phase or in the fluid similar to the mobile phase. On the other hand, the stationary phase is always placed in a column and can be composed of irregularly or spherically shaped particles. HPLC separation is performed under various conditions. The most frequently used parameters for quantification of venlafaxine and its metabolites by HPLC and other separation techniques are compiled in Tab. 2 – *next page*.

HPLC with UV detection was applied by Fonseca et al. [2] for the analysis of VEN and its metabolites in the rat liver. The assay involves the chiral HPLC separation under normal-phase mode of elution and detection at 230 nm. The column with a chiral cartridge and a mixture of hexane with 2-propanol (95 : 5, v/v) plus 0.025% diethylamine as a mobile phase were used for the resolution of enantiomers after their isolation from the liver with the aid of liquid-phase microextraction (LPME). In this extraction, three different solvents are applied. In the first stage, the analyte is extracted from aqueous phase (the donor one, it is the diagnostic material) into organic one, and in the second stage, the extraction is carried out again into another aqueous phase (the acceptor one). The limit of quantitation (LOQ) for both analytes is equal to 200 ng/mL.

The above technique and the detector were also used by Matoga et al. [10], who analysed VEN and ODV in human blood. The chromatographic separation was performed on a C8 column at room temperature using acetonitrile with a phosphate buffer (30 : 70, v/v) as a mobile phase. Analytes were detected at 229 nm. For liquid-liquid extraction (LLE) of VEN and ODV from human blood, a mixture of isoamyl alcohol with hexane (1 : 99, v/v), and opipramole as the internal standard, were used. The LOQ was 100 ng/mL for ODV and 50 ng/mL for VEN.

Tournel et al. [11] have analysed not only VEN, but also other antidepressants, such as fluoxetine, citalopram, sertraline, paroxetine, milnacipran and fluvoxamine in human blood serum. For the analysis, HPLC with UV detection and clomipramine as an internal standard were used. The compounds were analysed using the C18 column as a stationary phase and a mixture of acetonitrile with a phosphate buffer (50 : 50, v/v). The analytes were detected at 200.4 nm. Prior to the analysis, the compounds were extracted from serum by LLE with a mixture of chloroform, 2-propanol and *n*-heptane (960 : 14 : 26, v/v/v). Under these conditions the LOQ for VEN is equal to 25 ng/mL.

Another kind of detection with a fluorimetric detector, was applied for the HPLC analysis by Vu et al. [1], who quantified VEN and its main metabolite in human blood plasma. A mixture of isoamyl alcohol and hexane (7.5 : 92.5, v/v) was used for extraction, whereas maprotiline was applied as the internal standard. Isocratic separation was carried out by utilizing a reverse-phase butyl-bonded column as a stationary phase and a mixture of acetonitrile and phosphate buffer (50 : 50, v/v) as a mobile phase. Detection of the analytes was at 276 nm (excitation) and 598 nm (emission). The limit of detection (LOD) for VEN and ODV was equal to 1 ng/mL and 5 ng/mL, respectively.

Table 2. The conditions for the quantification of venlafaxine and its metabolites by different separation techniques

Detection	Biological material	Stationary phase	Mobile phase	Internal standard	Extraction procedure	Quantitation range (ng/mL)	Ref.
HPLC							
UV, 230 nm	rat liver	Chiralpak AD	hexane and 2-propanol (95 : 5, v/v) plus 0.025% diethylamine	–	LPME	200 – 5000	2
UV, 229 nm	plasma	C8	acetonitrile – phosphate buffer pH 5.5 (30 : 70, v/v)	opipramol	LLE	200 – 4000	10
UV, 200.4 nm	plasma	C18	acetonitrile – phosphate buffer pH 3.8 (50 : 50, v/v)	clomipramine	LLE	25 – 500	11
spectrofluorimetric exc 276 nm em 598 nm	plasma	C4	acetonitrile – sodium phosphate pH 6.8 (50 : 50, v/v)	maprotiline	LLE	1 – 1000	1
spectrofluorimetric exc 238 nm em 300 nm	plasma	C8	acetonitrile – phosphate buffer pH 6.8 with 0.25% triethylamine (25 : 75, v/v)	citalopram	SPE	1 – 1000	12
spectrofluorimetric exc 228, 240 nm em 308 nm	human urine, plasma, rat brain	C18	acetonitrile – 0.4% TMACl pH 4.0 (60 : 40, v/v)	–	PDMS	0.2 – 2000 a 2 – 50000 ng/g b 1 – 20000 c	13
spectrofluorimetric exc 276 nm em 598 nm	plasma	C18	acetonitrile – phosphate buffer – triethylamine (33.5 : 66.6 : 0.4, v/v/v)	maprotyline	CPE	10 – 800	14
coulometric	plasma	C18	methanol – phosphate buffer pH 4.8 (70 : 30, v/v)	paroxetine	CBA	0 – 200	15
mass spectrometry	blood	C18	acetonitrile – water with 0.6‰ formic acid and 30 mM ammonium acetate (65 : 35, v/v)	fluvoxamine	SPE	5 – 1000	16

table continued on next page

UHPLC							
mass spectrometry	plasma	C18	methanol – 10 mM ammonium acetate (85 : 15, v/v)	verapamil	LLE	0.2 – 200	17
LC							
mass spectrometry	liver, peripheral blood, bile, urine and vitreous body	C18	methanol – 0.05 M ammonia – tetrahydrofuran pH 10.0 (67.0 : 32.5 : 0.5, v/v/v)	trazodon	LLE	50 – 5000	8
mass spectrometry	plasma	Chirobiotic-V	tetrahydrofuran – 10 mM ammonium acetate pH 6 (10 : 90, v/v)	mexiletine	SPE	1 – 1000 nM	18
mass spectrometry	femoral blood in corpses	Chirobiotic-V	tetrahydrofuran – 10 mM ammonium acetate pH 6 (10 : 90, v/v)	mexiletine	SPE	10 – 40000 nM	19
GC							
mass spectrometry	blood, brain and hair	–	–	–	SPE	10 – 500 d 50 – 1000 ng/g e	20
Capillary electrophoresis							
DAD, 195 nm	serum	untreated fused silica capillary	TRIS – phosphate buffer pH 2.5 with γ-cyclodextrin	tramadol	LLE	25 – 500	21

[a] = plasma, [b] = rat brain, [c] = urine, [d] = blood, [e] = brain

This detection was also applied by Mandrioli et al. [12], who analysed VEN and its main metabolite, ODV, in the blood plasma of patients receiving fixed doses of the drug, 75 or 150 mg per day. They used solid-phase extraction (SPE) with the C1 columns and the mobile phase for HPLC determination was composed of acetonitrile and a phosphate buffer (25 : 75, v/v) plus triethylamine. A spectrofluorimetric detector with the excitation at 238 nm and monitoring the emission at 300 nm was used. Citalopram was applied as the internal standard. LOD and LOQ for both analytes were 0.3 ng/mL and 1.0 ng/mL, respectively.

A HPLC equipped with the spectrofluorimetric detector was used by Unceta et al. [13] for the analysis of VEN, fluoxetine and citalopram in urine and human plasma, and in the rat brain. Separation was performed on a C18 column with the aid of a mixture of acetonitrile and tetramethylammonium chloride (TMA-Cl) (60 : 40, v/v). Detection of the analytes was performed at two excitation wavelengths, 228 nm and 240 nm. The measurement of emission was at 308 nm. A stir-bar sorptive extraction (SBSE) was used for extraction of the analytes, and it was carried out in several steps. The first was based on the extraction of the analytes into the solid phase, which was a mobile sorbent coated with polydimethylsiloxane (PDMS) and then, thermal desorption of the analytes from the sorbent into the aqueous phase was performed. LOQs for ODV are equal to 0.5 ng/mL, 5.0 ng/g and 2.5 ng/mL for plasma, brain tissue and urine, respectively. In the case of VEN, LOQs are 0.2 ng/mL for

plasma, 2.0 ng/g for brain tissue and 1.0 ng/mL for urine.

Qin et al. [14] analysed VEN in human plasma by HPLC with spectrofluorimetric detection. For identification of the analyte, a reversed-phase C18 column and a mixture of acetonitrile with a phosphate buffer and triethylamine (33.5 : 66.6 : 0.4, v/v/v) as a mobile phase, were used. The detection was made at 276 nm (λ_{ex}) and 598 nm (λ_{em}) and maprotiline was used as the internal standard. The LOD for VEN was 2 ng/mL and LOQ was 10 ng/mL. It is interesting to note that the cloud point extraction (CPE) was applied for the extraction of VEN. In this technique, a surfactant is added to the sample, then it is heated up to a temperature at which separation of the surfactant's phase from the aqueous phase takes place. Next, the analyte is extracted from the aqueous phase into the surfactant's phase. A non-ionic surfactant, Triton X-114, (polyethylene glycol tert-octylphenyl ether) was used for CPE.

The next kind of detection, using a coulometric detector, was applied by Clement et al. [15], who analysed VEN and ODV in human blood plasma using SPE with columns containing a silicone material with carboxymethyl cellulose. Chromatography was performed using isocratic reverse-phase with the C18 column as a stationary phase and methanol with a phosphate buffer (30 : 70, v/v) as a mobile phase. The selected operating potentials for the detector and guard cell were 0.65 V, 0.95 V and 0.98 V as indicated by voltammetry. The LOD for VEN and ODV were 2 ng/mL.

A very important detector used in chromatography is a mass spectrometer. It is one of the most accurate and expensive detectors that measures the mass-to-charge ratio of charged particles. It is used for determining masses of particles, but also elemental composition of a sample and chemical structures of molecules. Mass spectrometer works by ionizing chemical compounds to generate charged molecules or molecular fragments and measuring their mass-to-charge ratio.

This detector was also used by Juan et al. [16], who analysed not only VEN in human blood plasma, but also fluoxetine, citalopram and paroxetine. In order to purify the samples prior to analysis, extraction with the use of hydrophilic-lipophilic columns (HLB1cc) was applied. The HPLC separation was performed on the C18 column, using a mixture acetonitrile and water (35 : 65, v/v) plus formic acid and ammonium acetate as a mobile phase. The analytes were ionized in the electrospray ionization ion source of the mass spectrometer and detected in an ion recording mode. Under these conditions LOD for VEN was established as 0.1 ng/mL.

ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

UPLC or UHPLC (*Ultra High Performance Liquid Chromatography*) is a modern type of the HPLC system that has been improved to work at a much higher pressure than HPLC. The particles size of the column packings are also smaller than in HPLC columns. This improvement enables a better resolution of the sample in a shorter time.

UPLC coupled with tandem mass spectrometry was applied by Qin et al. [17] for the quantification of VEN and ODV in human blood plasma. Prior to chromatographic separation, the sample was pretreated by a one-step liquid-liquid extraction with diethyl ether. The separation was carried out on the C18 column with a mixture of methanol and ammonium acetate (85 : 15, v/v). The detection was performed on a triple-quadrupole tandem mass spectrometer by multiple reaction monitoring mode *via* electrospray ionization source. As the internal standard, verapamil was applied and the lower limit of quantification (LLOQ) for VEN and ODV was 0.2 ng/mL.

Liquid Chromatography – Mass Spectrometry

LC (*Liquid Chromatography*) was applied by Goeringer et al. [8] for the analysis of VEN and ODV in the liver, peripheral blood, bile, urine and vitreous body, the materials originating in the majority of cases from suicides. For homogenization of the liver, ultrasound was used, and after incubation of the samples in a 10 M NaOH solution and isolation of the analytes with butyl chloride, they were analysed by the LC-MS technique. Chromatographic separation was achieved using the C18 column and a mix-

ture of methanol, ammonia and tetrahydrofuran (67.0 : 32.5 : 0.5, v/v/v). Mass spectral detection of the ions was achieved using atmospheric pressure electrospray ionization in a positive mode. LOQ for VEN and ODV was 50 ng/mL. During the study it was demonstrated that in the *postmortem* material, the VEN to ODV ratio in each of the analysed tissues was around 10:1.

Kingbäck et al. [18] quantified VEN, its metabolites and enantiomers in human plasma and in whole blood samples. They employed LC coupled with electrospray tandem mass spectrometric detector, and SPE was carried out on C8 columns. Chromatographic separation was performed on a chiral column with the aid of tetrahydrofuran and ammonium acetate (10 : 90, v/v) as a mobile phase. The detection was carried out by mass spectrometry with positive electrospray ionization. LLOQ for the enantiomers of VEN and ODV was 0.5 nM. The experiments have shown that HPLC with tandem mass spectrometry could be applied for the quantification of particular enantiomers, both in human plasma and in whole blood, contrary to the HPLC with UV detection which was less selective. Moreover, SPE assures a high recovery (above 75%), which allows to use smaller sample volumes (0.2 mL). This is especially important when the method is used for the analysis of whole blood, because its matrix can lead to quick blocking of the columns.

Kingbäck et al. [19] isolated VEN, ODV and their enantiomers from postmortem femoral blood of corpses by using C8 columns. These columns were washed first with water, then with a mixture of methanol and water, and finally with acetonitrile. The elution of analytes was performed with a mixture of acetonitrile and trifluoroacetic acid. For the chromatographic analysis, a chiral column and a tandem instrument equipped with an electrospray interface operating in the positive ion mode were used. The LLOQ was equal to 0.14 ng/g for enantiomers of VEN and 0.13 ng/g for enantiomers of ODV. The analysis by LC/MS/MS method has shown that the average VEN level in blood ranged from 0.1 to 1.0 μg/g. As a result of the investigation of VEN metabolism, it was found that the rate of this process was highly correlated with the CYP2D6 isozyme genotype of a patient.

Other separation methods

According to the literature, VEN and its metabolite are also determined by other separation methods, i.e. by gas chromatography (GC) and capillary electrophoresis (CE). First of them belongs to the chromatographic techniques in which gas is used as a mobile phase. In the other, the separation of compounds depends on a differential migration of analytes in an electric field. The analytes are separated based on their size-to-charge ratios.

From the toxicological point of view, it is crucial to determine the level of VEN and ODV in a postmortem material. Willie et al. [20] analysed 12 antidepressants in blood, brain and hair of suicides. Prior to isolation of the analytes, the brain samples were properly purified and hair samples were washed in order to remove external contaminations. Quantification of the analytes by the GC/MS technique has shown that it was possible to determine the level of VEN and ODV in the brain and hair even when it was impossible to do in blood samples. Moreover, it was noticed that the compounds were uniformly distributed in the brain, and therefore there was no need to isolate the analytes from a particular part of the brain. The analysis of hair provides information of VEN therapy over several previous years.

Capillary electrophoresis with a diode array detector (DAD) was also used for the quantitation of VEN, its main metabolite and enantiomers in human blood plasma by Rudaz et al. [21]. In the investigation, charged cyclodextrins were used which were added to the mobile phase (phosphate buffer). The separation of the compounds was carried out in an untreated fused silica capillary. The applied voltage was set at 20 kV and the capillary temperature was 25°C. The isolation of analytes was carried out by liquid-liquid extraction using a hexane – ethyl acetate mixture (80 : 20 v/v). The internal standard was tramadol hydrochloride. The LOQ for each enantiomer LOQ was equal to 25 ng/mL.

CONCLUSION

In conclusion it can be stated that the quantification of VEN and its metabolites enables moni-

toring of these active pharmaceutical ingredients in blood, and therefore optimizing their doses. The literature review on methods used for the determination of VEN and its metabolites in diagnostic materials shows that HPLC with UV, spectrofluorimetric, DAD or MS detectors is the most popular separation method. Two of them, UV and spectrofluorimetric detectors, are used most frequently. UV detectors are the most popular ones and they can be used in routine diagnostic tests.

The presented methods are enough sensitive for the detection and quantitation of VEN in all the studied biological matrices. They can be used for the quantitation of VEN and ODV in the diagnostic materials at the level that occurs during treatment. In all the cases, the limits of quantitation were lower than 70 ng/mL for VEN and 200 ng/mL for ODV. Moreover, for the analytical separation, a mixture of acetonitrile with phosphate buffer as a mobile phase and the C18 column as a stationary phase are usually applied.

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