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Sertraline – isolation methods and quantitation in biological material

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Summary

Sertraline (SRT) is a modern and relatively safe selective serotonin reuptake inhibitor often used in the treatment of depression. Monitoring the body levels of this drug and its active metabolite, N-desmethylsertraline (DSRT), permits optimizing the dosage and personalizing the treatment, especially in the case of severe adverse reactions or lack of response to the applied therapy. The determination of SRT and DSRT in diagnostic material, i.e., blood, plasma, urine and saliva, and also in biological material from deceased persons, requires a variety of sensitive and reliable analytical methods to determine both the total drug level (blood) as well as the level of the unbound form (saliva and urine). This paper presents a detailed literature review of the methods of SRT and DSRT isolation from biological material and analytical techniques used for their determination. These include extractive procedures such as solid-phase extraction and microextraction as well as liquid-liquid extraction. We pay particular attention to the parameters taken into account during optimization of extraction, i.e., the effect of pH, type of solvent and composition of the solvents mixture, on washing various types of sorbents (hydrophobic, hydrophilic-lipophilic and ion exchange) and elution of analytes. We show the advantages and disadvantages of the extraction techniques in terms of efficiency and precision of extraction. We also discuss protein precipitation as one of the more recent methods of sample purification. In our presentation of the final determination techniques, i.e., HPLC, LC and GC, we focus on the type of detector (UV, nitric-phosphate, MS) as the basic factor determining the sensitivity, expressed as the limits of detection and quantification achieved by a given method.

Key words: sertraline, sample purification, quantitative analysis

Introduction

Depression is one of the most common diseases in the world. Defined as a mood disorder with symptoms lasting at least two weeks, it is associated with feelings of sadness, dejection, lack of energy, unwillingness to act, sleep disorders, constant fatigue, as well as avoidance of social contacts. Depression also frequently results in

self-injury and suicide. According to the Centers for Disease Control and Prevention, antidepressants are one of the most frequently prescribed drugs in the United States. The main purpose of antidepressants is to improve mood as well as normalize the circadian rhythm and, consequently, improve the quality of life. Antidepressants are divided by generation and mechanism of action, with most of them working by increasing the concentration of monoamines, mainly serotonin, norepinephrine and dopamine in the synaptic space. They can also directly affect receptors, second messengers and transcription in the cell nucleus [1].

One of the most important groups of antidepressants are selective serotonin reuptake inhibitors (SSRIs), which include sertraline (SRT) as well as fluoxetine, paroxetine, fluvoxamine, citalopram and its enantiomer – escitalopram [2]. Their main mechanism of action is inhibition of the serotonin transporter located in the presynaptic membrane of the axon responsible for serotonin reuptake. In addition, SRT has a weak affinity for α_1-adrenergic receptors and to a small extent inhibits the dopamine transporter [1, 2].

Pharmacological properties of sertraline

After oral administration, SRT is significantly affected by first-pass metabolism. As a result of N-demethylation, its active metabolite, N-desmethylsertraline (DSRT) is formed. Further stages include oxidative deamination, reduction, hydroxylation and conjugation with glucuronic acid. The half-life of SRT is 26 h, and for DSRT is between 62 h and 104 h [3]. Five isozymes – CYP2B6, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 – are responsible for the metabolism of both compounds.

98% of SRT in plasma is bound to proteins, and the highest concentration occurs from 4.5 h to 8.5 h after oral intake. It is removed from the body in the urine and feces. In both cases, about 40% of SRT is excreted in the form of metabolites. From 12 to 14% of SRT is excreted in the urine in an unchanged form.

The main SRT metabolite, DSRT, has a low affinity for the serotonin receptor and therefore is not associated with any clinical effects. However, its concentration may indicate the speed of SRT metabolism and its interaction with other drugs, such as anti-coagulants, non-steroidal anti-inflammatory drugs, and β-blockers. Lower plasma levels of SRT and DSRT are usually seen in tobacco smokers. In turn, higher levels of these two substances are observed in older adults [4]. Monitoring drug concentration is recommended when using SRT in children and adolescents due to the higher probability of self-harm and suicidal behavior [5].

Due to the lack of interaction of SRT with food, it can be administered independently of meals. Dosage usually starts from 50 mg/day, then is gradually increased up to 200 mg/day [3]. The most common side effects, in at least 10% of patients treated with SRT, are from the effects on the CNS, such as insomnia, headaches, dizziness, fatigue, and drowsiness. Other common side effects are gastrointestinal disorders, such as nausea, diarrhea, indigestion or lack of appetite. The most dangerous side effect, known as the serotonin syndrome, results from excessive accumulation of serotonin in the brain, and manifests as muscle stiffness, hyperthermia, clonic convulsions, cardiac arrhythmia, arousal, hallucinations, delirium, and then coma. It is a serious and life-threatening

condition which should result in immediate discontinuation of treatment and the use of a serotonin antagonist and muscle relaxants [3].

Due to the penetration of SRT through the placenta, it is not recommended during pregnancy, although there are no unambiguous test results confirming a teratogenic effect. The use of SRT in the 3rd trimester of pregnancy may result in withdrawal syndromes in the newborn, such as hypotension or hypertension, apnea, cyanosis, seizures, fluctuating body temperature, hypoglycemia, food intake difficulties, increased muscle tone, as well as dyspnea and persistent pulmonary hypertension [3]. The results of a study on the use of SRT during pregnancy show that the plasma concentration in women in the 3rd trimester was 68% higher than in non-pregnant women. Similarly, DSRT concentration was also elevated, although the ratio of SRT to DSRT in pregnant women was slightly lower [6]. Another study aimed at finding a correlation between the daily dose of SRT and levels in blood serum of women during labor, as well as in the amniotic fluid and umbilical cord blood. A correlation was found between SRT dosage and levels in the amniotic fluid. However, no relationship was found between the level of SRT in the serum, amniotic fluid and umbilical cord blood, nor between the level of SRT in the amniotic fluid and umbilical cord blood [7].

The purpose of monitoring sertraline concentration

Although SRT is a modern and relatively safe medicine, frequent use of the drug, including in children, requires appropriate analytical procedures to control levels in the body. Taking into account the significant intra-individual and inter-individual variability in the concentration of SRT in the body, it is usually recommended to simultaneously determine the level of its main metabolite, DSRT. This is due to the fact that fluctuations in the concentration of SRT and DSRT at the same dose may differ by over 50% between individuals, whereas this difference decreases to 20% for the SRT/DSRT ratio [8]. The simultaneous determination of both compounds also allows monitoring of the interaction of SRT with other drugs, especially since it is metabolized by five isozymes. In addition, as SRT shows a weak inhibition of four isozymes, namely, CYP1A2, CYP2C8, CYP2C9, and CYP2D6, attention should be paid to the level of SRT at doses above 150 mg/day simultaneously with other drugs metabolized by these isozymes [9].

CYP2C19 is inhibited by proton-pump inhibitors (PPIs), medicines often used in the treatment of diseases of the digestive system. A study on the simultaneous use of PPI and SSRI showed that esomeprazole causes a significant (38.5%) increase in plasma SRT concentration [10]. This was especially evident in the elderly, over 80 years of age. Lowering the SRT dose is also indicated in the case of persons with the CYP2C19 isozyme mutation, i.e., 'poor metabolizers' [11]. It was found that after a single dose to healthy volunteers, the area under the AUC curve in slow-metabolizing subjects was 41% greater than in the case of extensive metabolizers. Similar observations were made by analyzing plasma SRT and DSRT levels in 211 patients aged 8–20 years at an average dose of 50 mg/day. Slower metabolism usually resulted from the genetic change in the allele encoding CYP2D6 activity [12].

Changes in CYP2C19 and CYP2D6 isozymes have also been observed during pregnancy, the former being significantly impaired by hormones. In addition, increased blood flow through the kidneys may result in a change in SRT pharmacokinetics, which usually results in the need to change the dosage of the drug, especially in the third trimester of pregnancy [13].

SRT levels in the body can be monitored with a number of SRT and DSRT determination methods in various biological materials. Often, they are also used to confirm or rule out the presence of SRT and DSRT. Therefore, after validation, analytical methods are used both to simultaneously determine SRT and other tested compounds in samples from people undergoing SRT therapy [4, 14–17], as well as samples from healthy people [18, 19]. In some cases, selectivity of the methods was confirmed and drug interactions in analyte isolation and chromatographic determination were excluded [4, 20]. Another important application of analytical methods is toxicological analysis, used to determine the causes of death [21].

Determination of the level of the drug in the body can also be used in so-called monitored therapy (therapeutic drug monitoring – TDM). Usually, its goal is to individualize patient therapy and dose selection to enhance the effect of the drug and minimize toxic effects. This is particularly important for substances with a narrow therapeutic index. In the case of SSRI, including SRT, TDM should be used when there is no improvement in the patient's health despite a properly applied therapy. Studies show that about 50% of patients do not reach an optimal concentration of the active substance in plasma after a single dose of the drug [22].

An important aspect of TDM is the reduction of patient treatment costs. In patients responding to therapy, it was possible to lower costs by 39% in relation to patients who did not respond to treatment. This also helped to improve patient quality of life [23]. In addition, the introduction of TDM in the treatment of depression in older adults with SSRI resulted in a reduction of 10.2% in treatment costs, mainly due to lower doses of the prescribed drugs [24].

Additionally, in the case of therapy with SSRI, TDM should be used in patients over 60 years of age due to the elevated blood level of the drug, often exceeding a therapeutic concentration [25]. However, statistical data indicate that TDM is used 15% less frequently in patients from this age group than in people under 60 years of age. With an increase in age, the difference was even greater, so that in patients over 90 years of age, TDM was conducted three times less frequently than in the case of people under 60 years of age, which is inconsistent with the basic assumptions of TDM.

The use of TDM in children and adolescents aged 8–18 enabled determination of the linear relationship between the dose of SRT and blood levels, as well as between a normalized dose adjusted to the patient's body weight and the level of the drug in the body [26]. Although no relationship was found between serum SRT concentration and therapeutic effect, age, sex or tobacco smoking in this age group, it was noticed that in the case of patients using SRT the severity of adverse reactions was proportional to the drug concentration in plasma. Adverse reactions were also significantly more frequent in the case of combination therapy.

The age and sex of adult patients, especially those over 60 years of age, have a significant impact on plasma SRT concentration, as confirmed by a study indicating high individual variability of plasma levels [27]. After dividing the plasma SRT concentration by the daily dose of the drug, it was observed then that in women over 60 years of age there was a strong correlation between the SRT dose and the plasma concentration.

Individualization of therapy and reduction of treatment costs are among the basic goals of TDM, implemented by determining the level of SRT and DSRT in the body. If several medicines are used at the same time, monitoring SRT levels in plasma can help detect the interactions between drugs. This is especially important when the patient has several conditions and each of them requires an individual therapy. Drugs may not only mutually affect the metabolism of each other, but can also enhance side effects. One such action is the reduction of pseudocholinesterase activity by SRT. Lower activity of this enzyme may result in a significant prolongation of action, e.g., in the case of mivacurium, a relaxant of striated muscles [28]. When SRT and methadone are used simultaneously, SRT may contribute to a small increase in serum methadone concentration in the first 6 weeks of treatment [29]. Therefore, it is advisable to monitor the concentration of methadone due to its reduced metabolism in these conditions.

Co-administration of antipsychotic drugs with SSRI may result in an increased likelihood of QT prolongation [30]. The most exposed to this effect are poor metabolizers, although there was no correlation between the frequency of this side effect and the dose of the antipsychotic drug.

Literature data show that the concentration of SRT in the blood after a single 50 mg dose in healthy subjects is 14.10±6.49 ng/mL [19]. Determination of SRT in autopsies showed that the reference therapeutic concentration of plasma SRT should be range from 6.1 to 76.0 ng/mL [21]. Literature data indicate that in people who have been on long-term treatment with SRT, the level of the drug in the blood fall within this mentioned range [4, 17]. In addition to SRT determination, in some cases the level of the main metabolite, DSRT, is also determined. Its blood concentration is higher than that of the parent compound due to its much longer half-life. The determined SRT and DSRT levels in patient blood samples were 31 ng/mL and 167 ng/mL, respectively [4]. The levels of SRT have also been compared in different biological materials, which makes it possible to determine drug excretion. One of the methods was used to determine SRT in blood and saliva samples obtained from patients treated with this drug. The material for analysis was collected at an interval of two weeks. It was found that the salivary concentration of the drug was less than 5% of its concentration in the blood. The blood levels in the first and second week were 13.1 and 27.1 ng/mL, respectively, while in saliva they were 0.7 and 1.1 ng/mL [15]. The level of SRT in the saliva of drivers stopped by the police was in the range of 22–24 ng/mL [18].

SRT has also been determined in the breast milk of patients [16, 31]. During one of the tests, milk samples were collected twice, once at the beginning and again at the end of feeding. It was found that the concentration of SRT in the initial sample was close to that in plasma, and was 29 ng/mL. At the end of feeding, it was almost twice as high (46 ng/mL), which may be due to the higher fat and protein in the milk [16].

The level of SRT and its main metabolite in the mother's milk and serum, as well as the serum of the child, were also determined. The mother's blood samples were collected 24 h after administration of the drug, while the baby's blood was collected 2–4 h after feeding. Both analytes were present in maternal serum at concentrations between 8 and 92 ng/mL for SRT and between 17 and 212 ng/mL for DSRT. DSRT also reached a higher concentration in the plasma of the babies. Both SRT and DSRT were present in all tested milk samples and the concentration of SRT in milk positively correlated with the dose of the drug [31].

Methods for the extraction of sertraline from biological material

The developed methods should enable the determination of SRT and DSRT in therapeutic ranges, which in the case of serum SRT are from 50 to 250 ng/mL. Studies carried out so far have indicated that after oral administration SRT achieves a higher concentration in young healthy women (mean concentration 166 ng/mL) than in young healthy men (118 ng/mL). In the case of DSRT, the level of analyte also varied depending on the sex (244 ng/mL in women and 156 ng/mL in men). In older patients, SRT and DSRT concentrations were also higher in women than in men, and were 147 ng/mL and 135 ng/mL, and 274 ng/mL and 237 ng/mL, respectively. The study also showed that on the 9th day after the end of treatment, SRT was detected in the urine, with a concentration of the unchanged form of the drug at 0.2% [32].

Usually, active substances are determined in the blood, which allows determining the concentration of unbound and protein-bound fraction of the drug. Blood, and – above all – plasma, are also the basic diagnostic materials in the case of SRT and DSRT. However, sometimes saliva may constitute a useful alternative to blood, allowing determination of the level of the unbound drug. When a given compound is strongly bound to blood proteins, the concentration of the unbound fraction in the saliva is low. This means that the content of the analyte in this biological material may be insufficient and its determination impossible. However, it should be remembered that it is the unbound fraction that is responsible for the drug's action. Its level is therefore an indicator of the current activity of the drug, and may also indicate the interactions with other simultaneously used medicines. It may also indicate problems with the metabolism of the drug, as well as liver or kidney damage.

Literature data show that SRT and DSRT isolation methods are usually based on two types of extraction from biological material, as illustrated in Table 1. The first one is solid-phase extraction, which requires the use of specialized equipment, but allows the isolation of both compounds and purification of biological samples using small amounts of organic solvents. Liquid–liquid extraction is another common method of isolation. Therefore, this paper presents a review of literature involving the use of both techniques for the extraction of SRT and DSRT from biological material.

Table 1. **Review of the methods used for the purification of biological material and the quantitation of sertraline**

Biological material	Sample purification	Analytical technique	Stationary phase / Mobile phase (v/v)	Linearity (ng/mL)	Recovery (%)	LOQ	Ref.
SPE							
Plasma	C2	HPLC-UV	C8 / acetonitrile: phosphate buffer (pH 3.0) with triethylamine (35:65)	7.5–250.0	>94	7.5	[4]
	C18-BSA	LC-MS/MS	C18 / ammonium acetate with 0.1% formic acid: acetonitrile (90:10)	5.0–325.0	-	5.0	[35]
	HLB	LC-MS/MS	C8 / ammonium acetate buffer: acetonitrile (20:80)	0.5–60.0	81.47	0.5	[36]
	Oasis MCX	SPE-LC-MS/MS	C18 / ammonium hydrogen carbonate gradient (pH 10): acetonitrile	10–1000	>99	10	[14]
	Oasis MCX	HPLC-MS	C18 / ammonium acetate gradient (pH 8.1): acetonitrile	1–500	>78	5	[38]
Plasma Saliva	Oasis MCX	LC-MS/MS	C18 / ammonium formate gradient (pH 3.0): acetonitrile	2–500	49–72	2	[15]
Saliva	Bond Elut Certify	GC-MS	Methylsilicone/helium	–	>46.5	18.6	[34]
	Bond Elut Certify	LC-MS/MS	C18 / ammonium formate gradient with 0.001% of formic acid: acetonitrile	5–200	92	8.7	[37]
Human milk	Oasis MCX	HPLC-ESI-MS	C18 / ammonium acetate gradient (pH 8.1): acetonitrile	5–500	>85	2	[16]
LLE							
Hair	Methanol	LC-ESI-MS	C18 / methanol gradient, water with 0.1% of formic acid (80:20): methanol, water with 0.1% of formic acid (90:10)	-	-	-	[5]
Serum	Heptane/toluene – isoamyl alcohol	GC-NPD	PTE/helium	20–400	>71.4	20	[39]
Serum	Butyl chloride	LC-MS	C18 / acetonitrile: methanol: ammonium acetate (60:20:20)	1.53–153	>90%	1.53	[10]
Whole blood	Ethylacetate: n-heptane	UHPLC-MS/MS	C18 / ammonium formate gradient (pH 10.2): acetonitrile	7.6–920	>82	7.6	[21]
Other extraction methods							
Saliva	Precipitation with acetonitrile	LC-ESI-MS	C18 / methanol gradient, water with 0.1% of formic acid (80:20): methanol, water with 0.1% of formic acid (90:10)	-	-	-	[5]

table continued on the next page

Plasma	SPME	LC-MS/MS	C18 / ammonium acetate gradient with 0.1% of formic acid: acetonitrile	5–325	-	0.05	[17]
	SPME	LC-UV	Lichrosphere 60 RP: Select B / phosphate buffer (pH 3.8): acetonitrile (57:43)	25–1200	>70	25	[40]
	SPME	LC-UV	C18 / phosphate buffer (pH 4.5): methanol (55:45)	10–1000	-	10	[41]
	Precipitation with acetonitrile	LC-MS/MS	C18 / mobile: ammonium acetate with 0.1% of formic acid: acetonitrile (30:70)	0.1–50	>88	0.1	[19]
	Precipitation with acetonitrile	LCMS/MS	C18 / ammonium acetate with 0.1% of formic acid: acetonitrile	2.5–405.0	-	2.5	[20]

Ref. – references

Solid-phase extraction

In solid-phase extraction (SPE), the nature of the analyzed compounds should be taken into account in order to select a suitable sorbent for sample purification. The sorbent should selectively bind the analytes and allow elution by suitable solvents or their mixtures.

Due to the nature of SRT, i.e., its lipophilicity that facilitates penetration into the CNS, hydrophobic sorbents [4, 33–35] or those with hydrophilic-lipophilic properties are usually selected for extraction [36]. In addition, SRT isolation from the biological matrix is also carried out using columns which – in addition to hydrophobic groups – contain modifications allowing ion exchange, which enables them to selectively bind weak bases on their surface [14–16, 18, 34, 37, 38], most often being drugs acting on the CNS. In addition, columns of this type have a very low affinity to water-soluble compounds, allowing a thorough purification of the sample and removal of the matrix.

Columns with C2 hydrophobic sorbent and other types of columns, e.g., those with HLB, C8 and C18 sorbents, were used by Mandrioli et al. [4] for the determination of plasma SRT and DSRT. Columns with a C2 sorbent allowed a good purification of samples with a precision of 3.9% at an accuracy higher than 90%. This type of column also provided good recovery, which exceeded 94%.

A recovery of SRT ranging from 91 to 118%, intra-day precision at 3.6% and inter-day precision of 7.4%, was observed in a method allowing the simultaneous analysis of 22 substances with antidepressant and antipsychotic activity [33]. The isolation of these drugs from human serum was performed with columns with a C18 hydrophobic sorbent. Recovery of the method for the remaining analytes ranged from 75 to 99%.

The hydrophobic C18 stationary phase was also modified by coating it with albuminous bovine antibodies in order to increase the rate of serum purification [35].

The optimization of the extraction process increased the sensitivity of the method and its selectivity, enabling the simultaneous determination of 16 psychoactive compounds. Intra-day and inter-day precision in the case of SRT determination was lower than 10%.

Plasma SRT has also been determined using hydrophilic–hydrophobic columns. The solutions eluted from the columns were injected directly onto the chromatographic column without prior evaporation and re-dissolution, to reduce the consumption of organic solvents and to increase the extraction efficiency. As a result, efficiency exceeded 81%. In addition, the stability of SRT was also determined both in plasma samples (before the extraction process) and after its isolation from the biological material. SRT only slightly decomposed during storage. The largest losses were recorded during long-term storage of the solution at – 70°C, when after 71 days the analyte concentration decreased by about 5% [36].

Determination of SRT and 13 other antidepressants together with their metabolites was performed with Oasis MCX type columns, which have groups selectively binding analytes with the properties of weak bases [14]. The advantage of the developed method was the use of a small volume of plasma (50 μL) and the possibility of direct application of extracts to the chromatographic column, a so-called on-line extraction without the need for evaporation and re-dissolution of samples. This method of analysis significantly increased the efficiency of extraction to over 99%.

The same type of columns was used to analyze 9 antidepressants, including SRT and their metabolites, in the saliva of healthy volunteers [15]. A rather low but acceptable recovery was obtained, which for the analyzed compounds ranged from 49 to 72%. The Oasis MCX columns also made it possible to eliminate the impact of the matrix on the determination of five SSRIs and their main metabolites in human blood plasma [38]. However, also in this case, the extraction efficiency for SRT was relatively low at 78%. The developed method was characterized by high intra-day and inter-day precision, not exceeding 6.5%.

Human milk is another biological material from which SRT has been isolated using MCX columns. Determination of drugs in this material is particularly important in the case of perinatal depression. Most often it is advisable to abandon breastfeeding so that no side effects occur in the newborn. The method for determination of SRT and other SSRI antidepressants as well as some of their metabolites in human milk was developed by Weisskopf et al. [16]. Before proper extraction, a 0.25 mL milk sample was deproteinized and then SRT was isolated by SPE. Validation showed that the method met the required criteria, i.e., the intra-day precision of determinations was 9.3%, and inter-day precision was 9.5%.

Columns with similar features, i.e., strong ion-exchange properties, were used to simultaneously determine 36 analytes in saliva. The goal was achieved thanks to the universal nature of the column sorbent and the use of fractionated elution [34]. The disadvantage of the method was the low extraction efficiency, which for SRT was between 46.5 and 74.9%.

Saliva was also used by Wylie et al. [37] for the simultaneous determination of 49 psychoactive substances. For their isolation, columns with ion-exchange properties

were used, with a sorbent modified with the addition of sodium sulphate to obtain high recovery – 92%.

Liquid–liquid extraction

Liquid-liquid extraction (LLE) allows isolation of compounds and simultaneous purification of the sample due to the different solubility of analytes in two immiscible liquids. In the case of compounds with high lipophilicity, such as drugs that pass to the CNS, their solubility in water and polar solvents is limited. Therefore, organic solvents are frequently used. Biological fluids, such as blood, saliva or urine, mainly consist of water, hence the use of LLE for the isolation of psychoactive compounds from biological matrices is fully justified.

LLE is characterized by a simplicity of execution and is also relatively fast. However, the high consumption of toxic organic solvents causes that it is less and less used. This type of extraction has been used to isolate SRT and four other psychoactive substances along with their metabolites from human saliva and hair [5]. After cleaning of the external impurities by repeatedly washing with methanol and drying, the properly collected hair sample was extracted with methanol in a Soxhlet apparatus. In the case of saliva, deproteinization was used, i.e., precipitation of protein with acetonitrile.

SRT along with other SSRIs and their metabolites have also been extracted from human blood serum [39]. Extraction was carried out with a mixture of heptane and isoamyl alcohol at a volume ratio of 98.5:1.5, and the extraction efficiency for the analyzed analytes ranged from 71.4 to 85.1% [39]. For SRT extraction from human serum, butyl chloride was also used, and the additional alkalization of the sample with sodium carbonate enabled the compound to be isolated with efficiency over 90% [10].

From the point of view of toxicology, methods for the determination of psychoactive substances in biological material from the deceased are extremely important. A mixture of ethyl acetate and n-heptane at a ratio of 80:20 (v/v) has been used for the determination of SRT in the blood of the deceased. In addition to SRT, 14 other substances were also isolated, including antidepressants and their metabolites, antipsychotics, analgesic, antihistamine, and β-blocker [21].

Other methods of sample purification

Isolation of analytes from biological material often involves a modification to SPE, namely, solid-phase microextraction (SPME). The basic difference between SPE and SPME is the use of a thin fiber with a sorbent applied, where the analyte is adsorbed from the matrix. The adsorbed analyte is then washed and evaporated, where it is then determined by gas chromatography (GC) or transferred to the mobile phase or other solution for desorption, and then introduced into the column of the liquid chromatograph (high-performance liquid chromatography – HPLC). This method of extraction has been used to analyze the concentration of SRT and other CNS-active

drugs in human plasma [17]. During the determination, silica gel modified with amino and butyronitrile groups was used. The influence of sorbent type on the extraction efficiency was also determined.

SPME with polypyrrole as a sorbent was used to isolate antidepressants from blood serum. Validation of the extraction process showed that the recovery was about 70% [40]. The same medications were also isolated from blood serum using a fiber coated with a mixture of C8 sorbent and a strong cation exchanger. Optimization of the extraction was based on determination of the effect of pH and the composition of the solvent mixture on the process of sorbent washing and elution of the analytes. The significance of the volume of the sample used for the analysis was also determined, and it was found that the optimal serum volume was 0.4 mL, while the sorbent washing was most effective with water acidified with 0.1% formic acid. In the optimized extraction conditions, the intra-day precision of the process was 8.7% [41].

One of the newest methods of sample purification, and also one of the simplest, is the precipitation of protein using organic solvents. Usually, a small amount of methanol or acetonitrile is added to the sample, which is then shaken and then centrifuged. The supernatant obtained in this way is injected on the column and subjected to chromatographic analysis. Precipitation can be used as a separate sample purification process [19, 20], or it can be a stage preceding proper extraction [16, 38].

The protein precipitation method was used to purify blood plasma samples to determine SRT. After the addition of acetonitrile to the blood, the sample was mixed thoroughly and then shaken and centrifuged. Finally, 0.01 mL of the supernatant was collected for the chromatographic analysis, obtaining a recovery over 88% for all tested concentrations [19].

Protein precipitation was also used to determine plasma SRT in addition to other antidepressants, antipsychotics, anti-epileptics, and sedatives. Also in this case, acetonitrile was used for the deproteinization of the samples; its volume was two times larger than the volume of the blood sample. 0.5 mL of the supernatant was evaporated and the dry residue was dissolved in a mixture of water with the addition of formic acid and acetonitrile (90:10; v/v). The intra-day precision of the developed method was lower than 12%, while its inter-day precision ranged from 0.1 to 10.4% [20].

Quantitative analysis of sertraline

The sensitivity of SRT determination methods, in the sense of detection and quantification, is to the largest extent determined by the type of used detector. The use of certain types of detection, e.g., mass spectrometry (MS), additionally confirms the identity of the analyte, which is not the case for an UV detector, a much less sensitive device compared to MS. However, the UV detector is universal and also incomparably cheaper than MS. This detector's appropriate sensitivity achieves a low limit of detection (LOD) and quantification (LOQ).

The quantitative analysis of SRT most often involves the use of liquid chromatography, both HPLC and LC, coupled with either a UV detector [4, 8, 26, 33, 40, 41] or

MS [5, 10, 14–21, 25, 35–38]. SRT has also been determined using GC coupled with MS [15] or a nitrogen-phosphoric detector [39].

The least favorable LOD and LOQ were obtained in the determination of SRT using the GC technique with a nitrogen–phosphate detector at 10 and 20 ng/mL, respectively, with the linearity of the method in the range of 20–400 ng/mL [39]. In contrast, the use of UV detection in HPLC provided LOQ at 4 ng/mL [26] and 5 ng/mL [33]. A similar LOQ for SRT and DSRT was determined by Mandrioli et al. [4], which for both compounds was 7.5 ng/mL. In the case of SRT, the limit of quantification was also the first point of the calibration curve. In contrast, for DSRT, the linearity of the method was checked in a concentration range of 10 to 500 ng/mL. LOD value was also determined for the tested compounds, which in both cases was 2.5 ng/mL. Significantly lower LOQ for both analytes was obtained using SPME extraction, which was determined at 25 ng/mL [40]. An appropriate modification of the sorbent allowed a reduction in LOQ to 10 ng/mL [41].

Literature data show that UV detection enables reaching an LOQ of several ng/mL, but it was not possible to achieve a value lower than 4 ng/mL. Also the lowest LOD was established at several ng/mL. Lower LOD and LOQ values can be achieved using mass spectrometry. However, it is not always necessary to strive for low LOD and LOQ values, especially when concentration of the analytes in the biological material reaches several hundred ng/mL.

The lowest LOQ for SRT was achieved with the LC technique coupled with MS, and it was 0.05 ng/mL [17]. However, the first point of the calibration curve was determined at 5 ng/mL. LOQ lower than 1 ng/mL was achieved in four cases; in three cases when mass spectrometry was coupled with LC, LOQ was 0.1 ng/mL [19] and 0.5 ng/mL [35, 36]. In the fourth case, ultra-high performance liquid chromatography (UHPLC) was used for the chromatographic separation, the limit was set at 0.76 ng/mL [21]. Most often, however, LOQ for SRT determined using LC coupled with MS, regardless of the extraction method or type of biological material, was determined at a level of several ng/mL [10, 14–16, 20, 36].

On the other hand, when determining SRT in saliva using GC coupled with mass spectrometry, the LOD and LOQ values were much higher – 6.2 and 18.6 ng/mL, respectively [34]. This may be due not only to the method of determination, but also to the type of biological material used for testing. Blood is the most often analyzed, but in this case saliva was analyzed, the composition of which differs from the composition of the blood. In addition, saliva samples often require additional purification steps, e.g., to remove mucins.

Conclusions

Determination of SRT and its metabolite in biological material of various origins allows primarily monitoring of the level of the drug in the body, which is extremely important in the case of severe side effects or lack of body response to the drug despite a proper therapy. Determination of SRT and DSRT allows optimization of the dosage and personalization of treatment. Another purpose for using quantitative analysis methods is rapid toxicological evaluation.

This presented review of literature on SRT extraction methods allows the statement that currently SRT is most often isolated from biological material using SPE, and an appropriate modification of the sorbent allows simultaneous isolation of several analytes with similar structure and action. This allows development of methods significantly reducing the use of toxic organic solvents and enabling the determination of analytes in possibly the smallest volume of biological material.

The cited literature also indicates the high popularity of hydrophobic adsorbents, which is related to the nature of the analyte. In addition, ion exchangers are also often used, which completely eliminate the effect of the matrix on the assay, but do not provide such extraction efficiency as hydrophobic sorbents. Among the analytical techniques used to determine SRT, the most important is liquid chromatography coupled with mass spectrometry. The use of this detector guarantees very good sensitivity of the method. However, it should be taken into account that this is the most expensive method, and the very low detection limits achieved by this are not always required in routine determinations. Therefore, the use of the DAD detector, a type of UV detector, enables simultaneous detection of a compound present in a small amount of sample and confirmation of its identity in a UV spectrum. This methodology of determination is more advantageous from the point of view of economics than the use of an expensive MS detector.

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