Validation of a Spectrophotometric Method of Some Antidepressant Drugs

Cadar Emin

Ovidius University of Constanta, Faculty of Pharmacy, Romania

Sirbu Rodica

University of Medicine, Pharmacy "Carol Davila", Romania

Ana Maria Ionescu

"Ovidius" University of Constanta, Faculty of Medicine, Romania

Anca Safta

County Clinical Emergency Hospital "Sf. Ap. Andrei", Constanta, Romania

Cezar Laurentiu Tomescu

Ovidius University of Constanta, Faculty of Medicine, County Clinical Emergency Hospital "Sf. Ap. Andrei", Romania

Abstract

Although the causes of depression include a wide range of conditions, from neurodegenerative to thyroid, they all have one common denominator, changes in the brain. Depression can be located in multiple brain regions simultaneously, which may be derived from a common molecular abnormality found in neurons in many brain regions. Abnormalities of receptors or impulse transmission could be the answer. In this work, a simple and accurate spectrophotometric method was developed for the analysis of escitalopram oxalate in various pharmaceutical formulations. In this method, absorbance was measured at 238 nm for escitalopram oxalate. Method validation parameters were studied. The method can be used for quality control of pharmaceutical forms containing escitalopram. Also, by the same method, the actual active substance concentrations compared to those listed on the package, the standard deviations and the relative standard deviations.

Keywords: validation of method, antidepressant drugs, spectrophotometric

Introduction

Depression can be translated as an imbalance at the level of certain neurotransmitters, the role of antidepressants being to increase the ratio of serotonin at the synaptic level. Depression can be due to totally unfortunate events that affect patients either at very young ages or those with major affective sensitivity, regardless of age. Serotonin also acts on other neurotransmitters, which activate brain areas responsible for mood and concentration. Major depressive disorder is a serious medical condition that is responsible for a considerable number of comorbidities. Despite decades of research, the neural basis for depression is still not fully understood. Evidence from neuroimaging, neuropsychiatry, and brain stimulation studies is explored to locate depression in the brain.

Also, the long-term effects of antidepressant drugs have not been studied. This paper presents a study on a pharmaceutical formulation that contains Escitalopram oxalates and which is presented in different commercial products. Certain general aspects that can generate depression are also presented. In order to apply an efficient treatment, the causes of this disease and the form of manifestation must be known.

The objective of the work is to carry out a study for the validation of a simple and precise spectrophotometric method for the analysis of Escitalopram oxalate.

Medical causes of depression

The medical and neurological causes of depression can be systematized in neurological, endocrinological, infectious and inflammatory causes and in various other causes such as cancer. Neuroimaging studies indicate that, despite the fact that many brain regions have been repeatedly implicated in the pathophysiology of depression, there is a notable absence of consistent findings to date. Recently, attention in neuroimaging has shifted from abnormalities at the regional level to those at the level of intraneuronal connections. Neuropsychiatric studies of Parkinson's disease and stroke provide clues to areas involved in depression. Similarly, stimulation of a variety of regions has been reported to be effective in treating depression. The nature of the distribution of depression needs to be thoroughly investigated, the primary and secondary affected areas need to be identified, and new models to explain the complexity of mental functions await exploration.

The most used antidepressants belong to the class of selective serotonin reuptake inhibitors, with two major indications: depressive episode and anxiety disorders. In the case of mild forms of depression, psychotherapy is recommended as a first step, and for moderate and severe forms, medication plus psychotherapy. Contrary to popular belief, antidepressants are not addictive. The effects appear gradually and set in after a few weeks of daily administration over a period of at least six months. It should be noted that antidepressants do not make you happy, they make you feel more in control of your own dark thoughts.

Anxiety disorders and depressive disorders are often underdiagnosed in contemporary medical practice due to their placement in the secondary, tertiary, or even further plane, often not being taken into account as a response to externalizing imbalance family chemist (Kaplan et. al, 2001). Everything we feel is due to connections that occur between neurons and chemical mediators. It was Mclean who introduced the concept that the brain is composed of three different assemblies, radically distinct in terms of their underlying chemistry, structure and, in evolutionary terms, the so-called triune brain (Maclean, 1985). Regional cortical imaging showed abnormalities in each subdivision to investigate the location of depression in the brain. A decreased metabolism at the level of the prefrontal cortex, especially at the dorsolateral and dorsoventral level within the prefrontal cortex is often found in major depressive disorder (Rigucci et. al, 2009), (Kimbrell et. Al, 2002). Deficient perfusion in these regions is associated with a reduction in problem-solving skills, a tendency to act on negative emotions, and suicidal behaviour (Desmyter et. al, 2011). This finding was successfully used in the formulation of a therapeutic strategy in which the dorsolateral prefrontal cortex was stimulated using transcranial magnetic stimulation (George, 2010). Decreased metabolism and blood circulation in this region in depression could be combated with antidepressant treatment (Mayberg et. al, 2000).

Structural imaging of the cortex has suggested a diminished volume of the frontal lobe present in depression, as well as a diminished volume of the orbitofrontal cortex (Kumar et. Al., 2000), (Schweitzer et. Al., 2001), (Bremner et. Al. 2002). The anterior cingulate cortex has become a subject of study in the psychopathology of depression because reductions in cingulate gyrus metabolism have been recorded in familial depression, and studies by Mayberg have described abnormalities in the cingulate and dorsal gyrus in depressive disorder (Critchley, 2004). This anterior cingulate cortex has been shown to be functionally divided into dorsal and ventral parts. The dorsal part is involved in cognitive aspects of emotion, including the association between emotional stimuli with negative valences, while the ventral part (gyrus) shows an extensive bilateral connection with limbic regions such as the cerebral amygdala and dorsomedial thalamus, as well as with the areas that control mood, the medial prefrontal cortex and the lateral and medial orbitofrontal cortex (Etkin et. al., 2006).

The influence of the ventral anterior cingulate cortex on the hypothalamus, which controls the endocrine system. The insular cortex and especially its anterior subdivision are involved in the expression of emotions such as disgust, self-esteem, self-evaluation of internal visceral state (Critchley et. al., 2004) and in response to taste and smell stimuli. In depression, insular cortex activity has been enhanced in response to disgust-inducing stimuli (Surguladze et. al., 2010), to negative images (Anand et. al., 2005), and insular volume is correlated with depression scores (Sprengelmeyer et. al., 2011). The main subcortical limbic regions implicated in depressive disorder are the amygdala, hippocampus, and dorsomedial thalamus. Both

structural and functional abnormalities in these areas have been found in depression. A reduced hippocampal volume (Schweitzer et. Al., 2001) has been noted in depressed patients. Patients who relapse with treatment have been shown to have higher hippocampal volumes before treatment, while patients with lower hippocampal volumes were more prone to relapse (Sprengelmeyer et. al., 2011).

The medication administered in the treatment of depression is very diverse and still insufficiently studied. The association of various diseases with depression complicates treatment schemes.

This paper presents studies on a drug presented as a commercial product under the name of Escitalopram. The objective of the work is to carry out a study for the validation of a simple and precise spectrophotometric method for the analysis of escitalopram oxalate.

Material and Method

Escitalopram is a selective serotonin reuptake inhibitor. It is the pure S enantiomer of the bicyclic racemic phthalate derivative of citalopram. The chemical formula is S-(+)-1-[3-(dimethylamino) propyl]-1-(p-fluorophenyl)-5-phthalancarbonitrile oxalate. Several analytical methods have been described for the quantitative determination of this substance, such as LC-MS for escitalopram (Singh et. al., 2004).

As equipment, a UV-VIS spectrophotometer, Shimadzu model 1601, which was set at a bandwidth of 1.8 nm and a wavelength with an accuracy of ± 0.5 nm, with a pair of cells of quartz having an optical wavelength of 10 mm. A digital balance HR 200 (Afcoset) and an ultrasonic bath SW 45 were also used. The standard stock solution was prepared by dissolving 10 mg of escitalopram oxalate in 100 mL of reference solvent to obtain a stock solution with a concentration of 100 μg/mL of the drug. The standard stock solution was diluted with reference solvent, obtaining solutions with concentrations of 2-20 μ_g/mL. They were scanned in UV and the calibration curve was made taking into account absorbance and concentrations.

To prepare the stock solution, ten 20 mg escitalopram oxalate tablets and ten 10 mg tablets from three different other brands were triturated to a fine powder. The powder equivalent to one 20 mg and one 10 mg tablet was transferred into a separate 100 mL container. They were then dissolved in the reference solvent by sonication for 20 minutes. The final solution was diluted with reference solvent to yield a stock solution of 200 μg/mL for the first brand and 100 μg/mL for the three different other brands. These solutions were filtered through Whatmann filter paper and further diluted to obtain six replicates. These were analyzed using the spectrophotometer.

Results and Discussions

The establishment of the parameters for the validation of the method for determining the concentrations of escitalopram oxalates by the spectrophotometric method is based on first obtaining the UV-VIS spectra and then establishing a calibration curve

based on which the initial parameters for the validation of the method should be established. In Fig. 1 are shown the UV-VIS spectra obtained for escitalopram oxalate, which shows a maximum at the wavelength of 238 nm.

In Fig. 2 shows the calibration curve for escitalopram oxalate. The characteristics of the calibration curve are systematized in Table 1. The correlation coefficient of 0.9999 shows that a very good linearity was obtained in the concentration range 2-20 µg/mL.

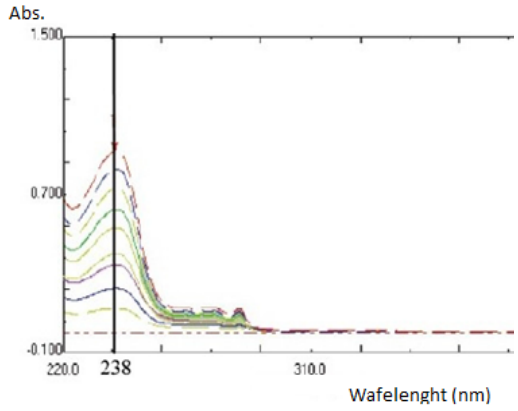

Fig.1 UV-VIS spectra for escitalopram oxalate

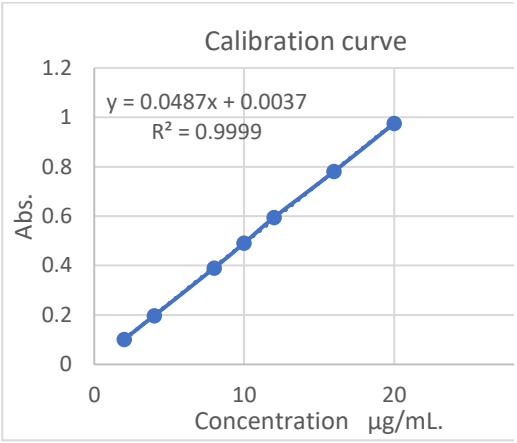

Fig.2 Calibration curve for escitalopram oxalate

Table 1 Characteristics of the calibration curve for escitalopram oxalate

Parameters	UV-VIS Spectrophotometric Methods
The regression equation	Y=0,0487x-0,037
Slope (b)	$48{,}7x10^{-3}$
Intercept (a)	$3.7x10^{-3}$
Linearity (μ_g/mL)	2,003-20,032
Correlation coefficient (r)	0,9999

Table 2 shows the results obtained for the validation parameters of the studied spectrophotometric method. All parameters were analyzed in compliance with the validation standards from the SR-ISO 8466/1990 series and the methodologies in force (Rodica Sîrbu et. al., 2017).

Table 2 Parameters obtained for method validation

Parameters	Result Escitalopram oxalate
Specificity	No interferences other than excipients were found
Linearitaty (correlation coefficient r)	0,9999

Accuracy*(% recovery)	99,72% 99.55% 99.69% and 99,98%
Relative Standard Deviation	0.3105
RSD% Accuracy (Precision)	0.616
Repeatability (n=6)	0,903
Over the course of a day (n=3)	0,176
Over consecutive days (days=3)	0,179
Lower limit of detection (LOD)(μg/mL)	0,191
Minimum limit of quantification (LOQ) (μg/mL)	0,637

*Average of three determinations

To study the validity and reproducibility of the method the recovery of the drug was studied which was determined at 80, 100 and 120% levels. A percentage recovery of 99.72% 99.55% 99.69% and 99.98% was made calculated for all brands. The percentages greater than 99% together with the low standard deviation justify the accuracy of the method. The relative standard deviation of 0.3105 also justifies the good accuracy of the method.

In order for the proposed method to ensure the precision required for an analytical determination, the acceptance criterion is considered met when for each concentration level, 0.616 is obtained, i.e. RSD% < 1%. In the present study, this criterion was fulfilled.

The LOD and LOQ were determined based on the standard calibration curve. To be estimated, the diluent was scanned under the UV region six times and the signal-to-noise ratio was determined. The LOD (0.191 μg/mL) and LOQ (0.637 μg/mL) were regarded as amounts for which the ratio was 3:1 and 10:1, respectively. Confirmation of accuracy is achieved by studying repeatability. This criterion demonstrates the closeness of the measured values to each other, for a number of measurements, obtained under the same conditions. To confirm the accuracy, it is demonstrated that applying the method, repeatedly, for the same samples, generates similar results.

In Table 3, the pharmaceutical formulations (brands) containing Escitalopram are systematized with the most important uses.

Table 3 Systematization of the analyzed drugs according to their uses

Escitalopram Brand	Uses
Escitalopram ATB 20 mg COM	Treatment of episodes of major depression, Treatment of panic disorders accompanied or not by agoraphobia. Treatment of social anxiety disorders (social phobia). Treatment of generalized anxiety disorders. Treatment of obsessive-compulsive disorders.
Escitalopram Aurobindo	Contains the active substance escitalopram. Escitalopram Aurobindo belongs to a group of antidepressant medicines called selective serotonin reuptake inhibitors (SSRIs).

	It acts on the serotonergic system in the brain, by increasing the concentration of serotonin. It is used to treat depression (major depressive episodes) and anxiety disorders (such as panic disorder with or without agoraphobia, social anxiety disorder, generalized anxiety disorder and obsessive-compulsive disorder).
Escitalopram Actavis	It belongs to a group of antidepressant drugs called SSRIs (selective serotonin reuptake inhibitors). It acts on the serotonergic system in the brain by increasing the concentration of serotonin. Disorders of the serotoninergic system are considered an important factor in the onset of depression and similar conditions.
Cipralex contains the active substance escitalopram.	It belongs to a group of antidepressant drugs called selective serotonin reuptake inhibitors (SSRIs). It acts on the serotonergic system in the brain by increasing the concentration of serotonin. It is used in the treatment of depression (major depressive episodes) and anxiety disorders (such as panic disorders with or without agoraphobia, social anxiety disorders, generalized anxiety disorders and obsessive-compulsive disorders).

Table 4. The results obtained and compared with the labels proposed by the brands

Brand	Amount on label (mg)	Amount found*(mg)	% of amount on label	Producer
Escitalopram Atb20	20	19,82±0,022	99,43±0,295	ANTIBIOTICE S.A. - Romania
Escitalopram Aurobindo 10 mg	10	9.95±0,042	99,35±0,275	AUROBINDO PHARMA ROMANIA S.R.L. - Romania
Escitalopram Actavis	10	9.97±0,012	99.69±0,195	ACTAVIS LTD. - Malta
Cipralex	10	9,91±0,032	99,53±0,325	H. LUNDBECK A/S - Danemarca

*Average of six estimates

Table 4 shows the results obtained by applying our validated method. It is found that the results show a good agreement between the active substance levels found in practice and the inscriptions on the product labels. The obtained results are in accordance with the new methodologies used in pharmaceutical control (Rodica Sîrbu et. al., 2017).

Conclusion

The study carried out allows us to highlight the following conclusions:

- Although the causes of depression include a wide range of conditions, from neurodegenerative to thyroid, they all have a common denominator, changes in the brain. These areas include the ventral tegmental area, responsible for the motivation-reward complex, and the insular and anterior cingulate cortex areas associated with psychological pain and suffering.
- Depression can be located within multiple brain regions simultaneously which may be derived from a common molecular abnormality found in neurons in many brain regions. Abnormalities of receptors or impulse transmission could be the answer.

In order to establish a correct diagnosis, the specialist doctor will have to approach the patient in order to obtain data from his history, such as anhedonia, lack of ambition, decreased energy level, social isolation, as well as data from the observation of the present mental state and somatic projections.

As for treatment, it includes both pharmacological and psychotherapy measures. Selective serotonin reuptake inhibitors (SSRIs) are the most prescribed first-line drugs in the treatment of depression, thanks to their safety, effectiveness and increased tolerability.

A simple and accurate spectrophotometric method for the analysis of escitalopram oxalate was developed in the paper. In this method absorbance was measured at 238 nm for escitalopram oxalate. The method can be used for quality control of pharmaceutical forms containing escitalopram. Escitalopram showed linearity at a concentration between 1-20 μg/mL, with a correlation coefficient of 0.9999.

Also, by the same method, the actual concentrations of active substance compared to those listed on the packaging, the standard deviations and the relative standard deviations both during one day and over several consecutive days were revealed.

Future studies should emphasize on studying in more detail various spectrophotometric methods for different drugs.

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